## TdT Assay

| pool | per tube | | final concentration |
|---|---|---|---|
| 25λ | 2.5λ | 1M K⁺cacodylate, pH 7.0 | 100mM |
| 5λ | 0.5λ | 50mM $CoCl_2$ | 1mM |
| 10λ | 1λ | 1:100 dilution of 0.5M DTT | 0.2mM DTT |
| 70λ | 7λ | ~~poly d(A) 100 50~~ | ~~≈ 6 μM 3'(OH) .014%~~ |
| 50λ | 5λ | 5mM dGTP (1:10 in label) | 1mM dGTP |
| | 9λ | $H_2O$ ($^3H$) | |
| | 1λ | enzyme, straight or diluted | |

Should be 50mM

| | TdT #1 (many very small vials) | TdT #2 (large screw top vial) |
|---|---|---|
| and 1 enzyme added | .01λ ⇒ "1-.01" | .01λ ⇒ "2-.01" |
| | .1λ ⇒ "1-.1" | .1λ ⇒ "2-.1" |
| | 1λ ⇒ "1-1" | 1λ ⇒ "2-1" |

1. Reaction incubated at 37°C for one hour

```
          USER  1

SR  CH  12     1 TIMES
CR             1 TIMES
SCR =OFF
AUC =OFF
SF  =ON
CHLC=          1
PST =      10.000 MIN
CH 1        2.000 2 SIGMA %
              .0 LSR
              .0 BKG
             .00 2 SIGMA B
               0 LL
             39% UL
CH 2        2.000 2 SIGMA %
              .0 LSR
              .0 BKG
             .00 2 SIGMA B
             555 LL
             940 UL
POS      CH 1    2S%      CH 2    2S%   TIME

150 1-1  823.2   2.2      10.3  14.6   10.00
151 1-1  748.7   2.3       9.6  20.3   10.00
152 1-.01 711.8  2.3      11.2  18.8   10.00
153 2-1  542.2   2.7      13.7  17.1   10.00
154 2-1  424.1   2.0      11.5  18.6   10.00

         623.4   2.5      21.8  13.5   10.00
```

These calculations have many errors, see p. 10

$\$2000/\text{mg} \rightarrow \$2000/\mu\text{g}$ (1 mM) $= 2000/\mu\text{g}/\text{l}$

true molarity of DNA  A (mw) ≅ 347 g/mole

$\frac{(2.5\mu\text{g} \times 10^{-6}\ \text{g}/\mu\text{g})(8 \times 10^{-3}\ \text{mol})}{(100)(347\ \text{g/mole})} =$  poly(A)$_{100}$ (mw) = (347)(100)

(should be 7!)  (should be 50)

$360 \times 10^{-13}$ moles

$\frac{360 \times 10^{-13}\ \text{moles}}{25 \times 10^{-6}\ \text{l}} = 1.44 \times 10^{-8}\ \text{M} = .044\ \mu\text{M}$

# G's added

$(1 \times 10^{-3}\ \frac{\text{M}}{\text{l}})(25 \times 10^{-6}\ \text{l})\ N = $ # molecules of G  N = Avogadro's number

$(1.44 \times 10^{-8}\ \frac{\text{M}}{\text{l}})(25 \times 10^{-6}\ \text{l})\ N = $ # molecules of 3' OH ends

max possible incorporation of G $= \frac{(1 \times 10^{-3})(25 \times 10^{-6})N}{(1.44 \times 10^{-8})(25 \times 10^{-6})N} = 9 \times 10^{-3} = 69{,}444$ G's per 3' end

data were used 1-1-1 and 1-1-2

actual incorporation  $\frac{800\ \text{cpm}}{(1360\ \text{cpm})(50)/250} = .0118$

(pool dot) (dil. of pool for spot)

$(.0118)(69{,}444) = 819$

If 2000 cpm  $\frac{2000\ \text{cpm}}{(1360)(50)} = .0294$

$(.0294)(69{,}444) = 2042$

$(\frac{1}{50})($

1-1-021 T=010.00 A=002094.0(1.5%) B=002556.8(1.5%) C=002600.1(1.5%) R=1.
1-1-022 T=010.00 A=000778.2(3.0%) B=000972.7(3.0%) C=001030.8(2.0%) R=1.
1-1-023 T=010.00 A=000151.4(7.0%) B=000187.7(5.0%) C=000214.1(5.0%) R=1.
1-1-024 T=010.00 A=000048.3( 10%) B=000060.9( 10%) C=000083.6(7.0%) R=1.2
1-1-025 T=010.00 A=000034.4( 15%) B=000043.6( 10%) C=000069.8( 10%) R=1.2

1-2-026 T=010.00 A=000029.5( 15%) B=000039.3( 15%) C=000063.7( 10%) R=1.3
1-2-027 T=010.00 A=000028.1( 15%) B=000033.0( 15%) C=000059.1( 10%) R=1.47
1-2-028 T=010.00 A=000031.9( 15%) B=000041.1( 10%) C=000054.7( 10%) R=1.32
1-2-029 T=010.00 A=000387.7(5.0%) B=000412.6(3.0%) C=000443.7(3.0%) R=1.25
1-2-030 T=010.00 A=000155.3(5.0%) B=000192.5(9.0%) C=000285.1(5.0%) R=1.20

2-1-031 T=010.00 A=000023.2( 15%) B=000030.4( 15%) C=000056.9( 10%) R=1.30
2-1-032 T=010.00 A=000025.7( 15%) B=000034.9( 15%) C=000059.5( 10%) R=1.
2-1-033 T=010.00 A=000030.1( 15%) B=000036.8( 15%) C=000061.7( 10%) R=1.20
2-1-034 T=010.00 A=000026.1( 15%) B=000034.7( 15%) C=000052.2( 10%) R=1.30
2-1-035 T=010.00 A=000028.8( 15%) B=000036.4( 15%) C=000061.4( 10%) R=1.85

2-2-036 T=010.00 A=000030.9( 15%) B=000039.6( 15%) C=000055.8( 10%)
2-2-037 T=010.00 A=000112.5(7.0%) B=000140.9(7.0%) C=000157.7(5.0%)

2-2-038 T=010.00 A=000086.6(7.0%) B=000114.9(7.0%) C=000133.7(7.0%) R=1.
3-1-1 039 T=010.00 A=000026.0( 15%) B=000036.6( 15%) C=000058.8( 10%) R=
3-1-2 040 T=010.00 A=000022.3( 15%) B=000031.6( 15%) C=000054.1( 10%) R=1.4

3-01-1 041 T=010.00 A=000024.4( 15%) B=000035.6( 15%) C=000058.8( 10%) R=1.45
3-01-2 042 T=010.00 A=000030.3( 15%) B=000042.9( 10%) C=000065.5( 10%) R=1.400
3-001-1 043 T=010.00 A=000020.5( 15%) B=000031.7( 15%) C=000055.2( 10%) R=1.550
3-001-2 044 T=010.00 A=000022.7( 15%) B=000033.0( 15%) C=000056.4( 10%) R=1.500
C1 045 T=010.00 A=000025.4( 15%) B=000034.7( 15%) C=000059.1( 10%) R=1.360

C2 046 T=010.00 A=000026.5( 15%) B=000039.8( 15%) C=000064.4( 10%) R=1.500
1-001-1 047 T=010.00 A=001352.4(2.0%) B=001717.4(2.0%) C=001788.6(2.0%) R=1.25
1-001-2 048 T=010.00 A=001362.6(2.0%) B=001712.0(2.0%) C=001733.7(2.0%) R=1.2

corrected for error on p. 2

## Unit Calculation - Larry's Way

1mM dGTP = $\frac{10}{1\text{n}M/\lambda}$

10λ of a 1:100 dilution of pool = 0.1λ spotted
spotted

this produced 1360 counts

~~13,600~~ cpm/nmole
1,360

~~13600 cpm/nmole~~

Assumed highest (+2) → $\frac{2000 \text{ cpm}}{\text{~~13,600~~ cpm/nmole}}$ = $\frac{1.5}{\text{~~1.5~~}}$ nmole incorporated
1,360

$\frac{1.5}{\text{~~1.5~~}}$ units/λ

$\frac{1360 \text{ counts}}{.1\lambda} \quad \frac{10\text{n}M}{\lambda}$

$1360 \frac{\text{CPM}}{\text{~~counts~~}} \cdot \frac{\lambda}{10\text{n}M} = 1360 \frac{\text{CPM}}{\text{n}M}$

pUC19 1µg/λ

SphI ≡ P-S

5λ pUC19 (=5µg)
2λ SphI (=10 units)
25λ 10x Low Salt buffer
15.5λ $H_2O$
25λ total

≡ λ-S .6µg/λ

1λ λDNA (600µg/ml)
1λ split (=5units)
2.5λ 10x L. Salt buffer
20.5λ $H_2O$
25λ total

HindIII ≡ P-H

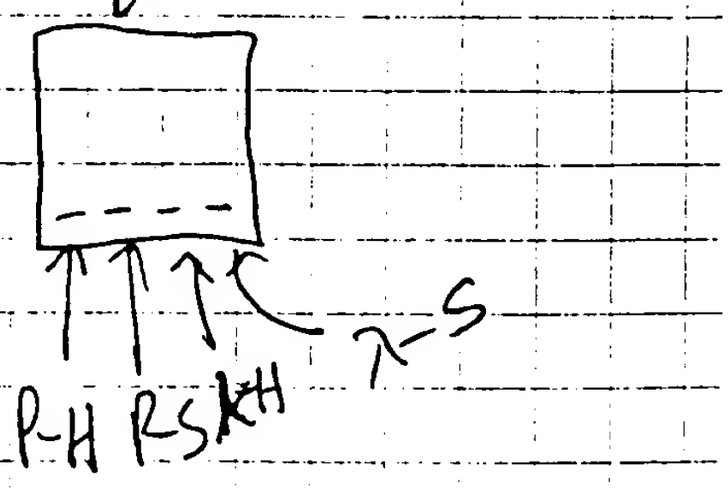
5λ pUC19 (=5µg)
2λ HindIII (=8 units)
2.5λ 10x "cut" buffer
15.5λ $H_2O$
25λ total

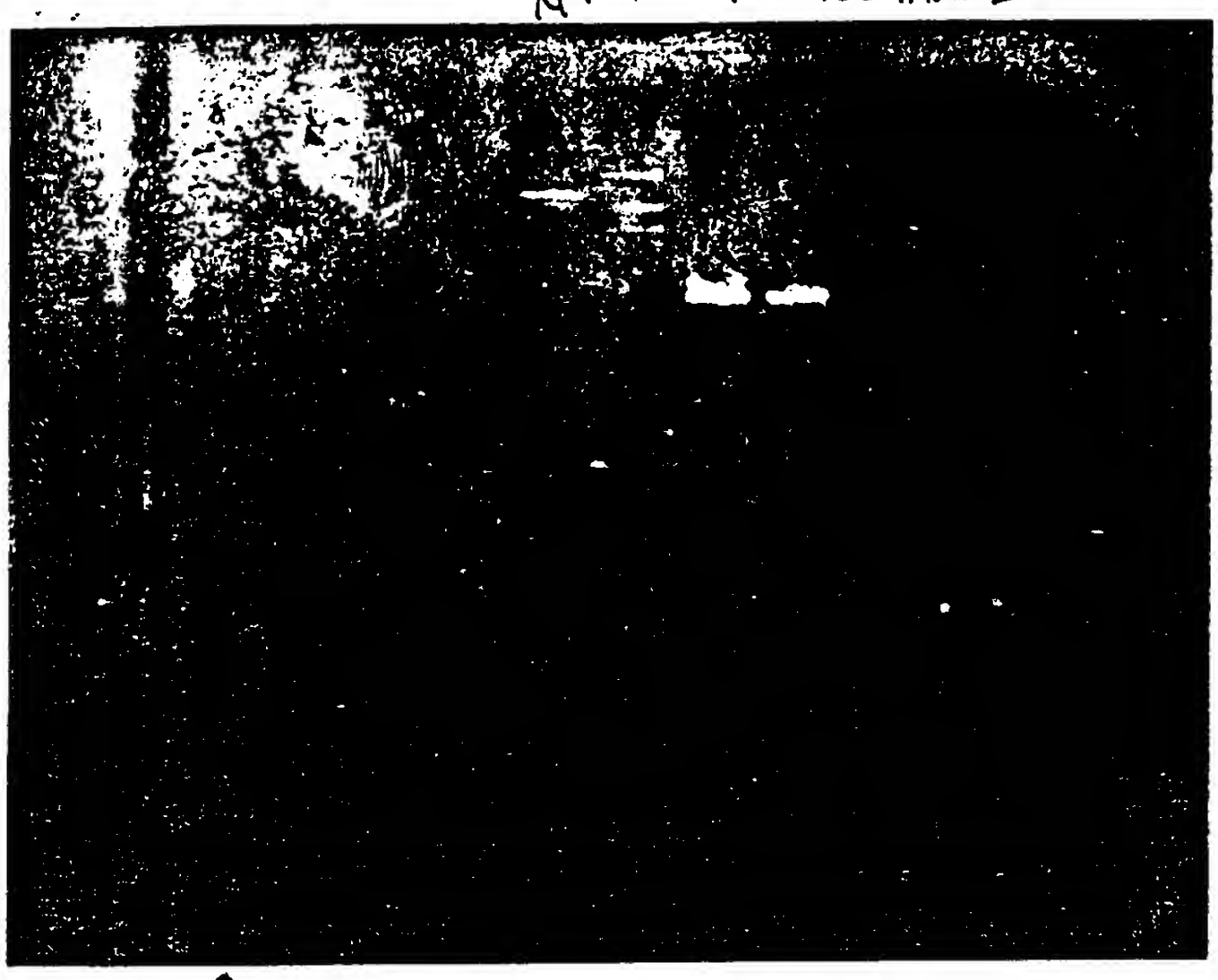
≡ λ-H

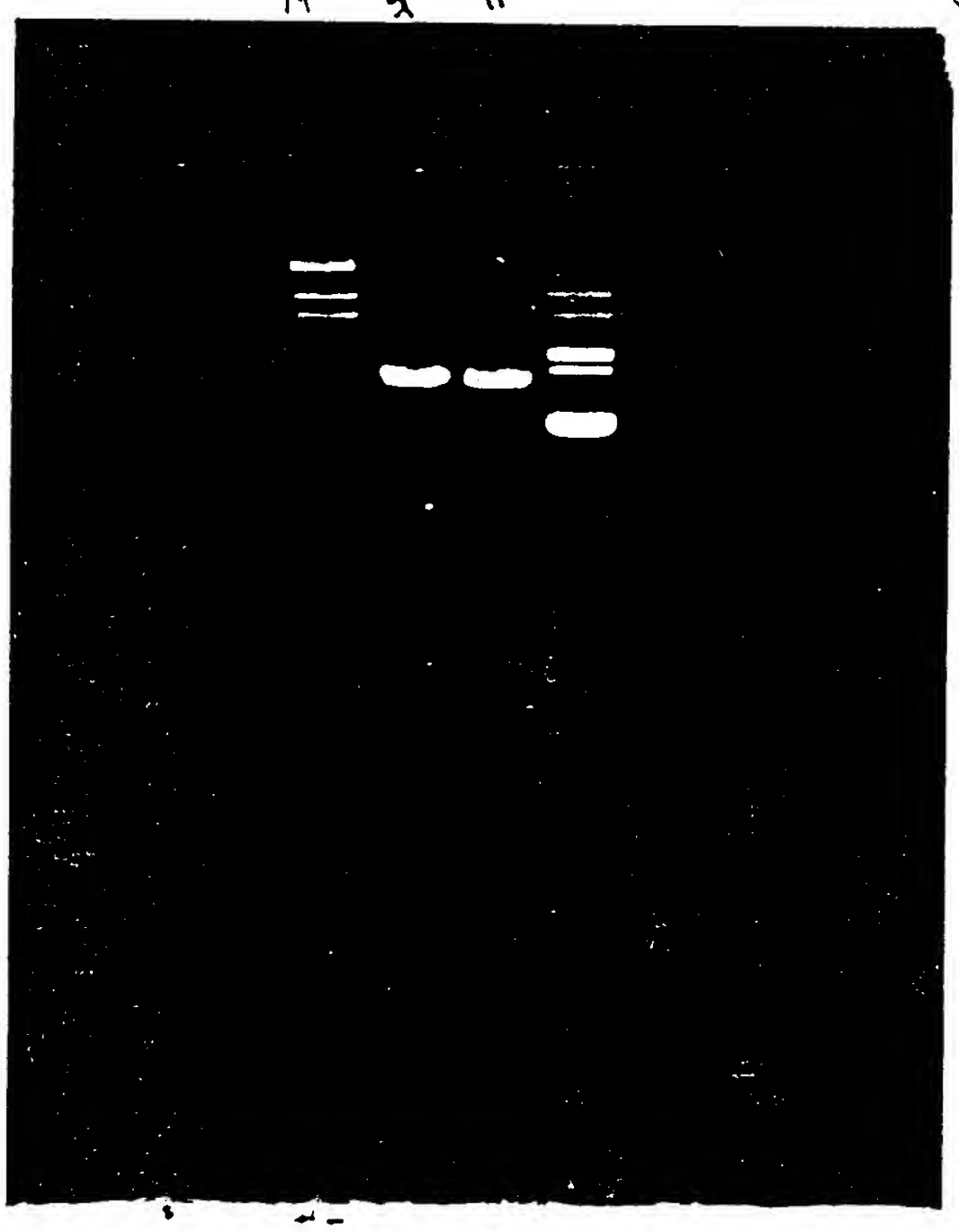
1λ λDNA
1λ HindIII (=4 units)
2.5λ 10x "cut" buffer
20.5λ $H_2O$
25λ total

37° at 10:15

Gel
TBE (diluted from 10x buffer)
.7% agarose




λ/SphI
λ/HindIII
pUC-SphI
pUC-HindIII


λ/HindIII
SphI
HindIII
pUC Alone

Phenol extraction & EtOH ppt of DNA

1. dilute DNA to 200λ w/ TE

2. Equilibrate Phenol w/ TE & adjust pH to 8.0 w/ 10N N

3. Add 200λ (equal volume) phenol

4. Spin & suck off phenol (bottom of 2 phases)

5. Spin again & again suck off phenol

6. add 500λ diethyl ether and let evaporate in hood (but mix first) to remove leftover phenol

7. ppt w/ 22λ 3M NH4 acetate
550λ of 95% EtOH

8. ppt @ -70°C, pellet 5' in Mfuge, drain & wash ppt w/ cold 70% EtOH and vac dry

eluted in 15λ TE

# TdT Assay using Rxn 19

enzyme fuse pool (glove first / blank dry)

100

| pool | per tube | |
|---|---|---|
| 250λ | 2.5λ | cacodylate |
| 50λ | 0.5λ | $CoCl_2$ |
| 100λ | 1λ | 1:100 dilution of DTT |
| 500λ | 5λ | dGTP (1:10 in water) |
| | 1λ | enzyme, undiluted |
| 450 dGTP | 2λ | DNA (=.8μg) @ .27 μg/1λ |
| 50 dTTP | 13λ | $H_2O$ |
| | 25λ total | |

Pool = 9λ/tube

↳ this amt is roughly scaled up from the poly(T)100 (multiplied by 50 (=2×25) since this DNA is ds & about 25× heavier — but it does have 2 ends upon which to add)

2 trials each of pUC/HIII and pUC/SphI
+ control of pool + $H_2O$

```
PROG  6        USER  1

SR  CH  12     1 TIMES
CR             1 TIMES
SCR =OFF
AQC =OFF
SF  =ON
CHLC=          1
PST =      10.00 MIN
CH 1        2.00 2 SIGMA %
              .0 LSR
              .0 BKG
             .00 2 SIGMA B
               0 LL
             397 UL
CH 2        2.00 2 SIGMA %
              .0 LSR
              .0 BKG
             .00 2 SIGMA B
             655 LL
             940 UL
```

| POS | | CH 1 | 2S% | CH 2 | 2S% | TIME |
|---|---|---|---|---|---|---|
| 139 | HindIII-1 | 27.7 | 12.0 | 9.2 | 20.8 | 10.00 |
| 140 | HindIII-2 | 29.0 | 11.7 | 9.2 | 20.8 | 10.00 |
| 141 | SphI-1 | 32.8 | 11.0 | 7.5 | 23.0 | 10.00 |
| 142 | SphI-2 | 35.2 | 10.6 | 9.5 | 20.5 | 10.00 |
| 143 | Control | 21.5 | 13.6 | 11.4 | 18.7 | 10.00 |
| 144 | Pool | 4575.3 | .9 | 7.7 | 22.7 | 10.00 |

↳ 10λ of 1:100 dilution

TdT Assay

9λ Pool
7λ poly(A) 100
1λ enzyme
8λ $H_2O$
25λ

as positive control

9λ Pool
1λ enzyme HindIII
4λ Puc
11λ $H_2O$
25λ

SphI

↳ 10:30 pm

Rxn stopped after 1/2 hour and ppt O/N in cold room

TdT Assay

| pool | per tube | | final concentration |
|---|---|---|---|
| 100λ | 25λ | cacodylate | 100mM |
| 20λ | 0.5λ | $CoCl_2$ | 1mM |
| 40λ | 1λ | 1:100 dilution of Oitt | 0.2mM |
| 280λ | 7λ | poly(A) | ~~2mM 3'OH~~ .04 |
| 200λ | 5λ | d6TP (1:10 in label) | 1mM d6TP |
| | 8λ | $H_2O$ | |
| | 1λ | enzyme, straight & serially diluted | |

3 batches of enzyme were tried at 1λ, .1λ, .01λ, and .001λ each. Batch 1 was already sorted into small vials. Batch 2 was in the large tube w/ a cap. Vial 3 was uncapped and covered w/ parafilm.

"A-B-C" ⇒ A = batch # (1, 2 or 3)
B = quantity of enzyme used (1λ, .1λ, .01λ, or .001λ)
C = trial number (1 or 2; two trials of each possible used)

C1 = Control #1 } pool w/o enzyme in same amt
C2 = Control #2 } per tube (25λ - 1λ = 24λ)

pool 1 } 10λ of a 1:100 dilution of the pool spotted
pool 2 } on a filter and dried

TdT Assay

poly(A)
9λ pool
2λ poly(A) 100
1λ enzyme
17λ

Puc/HIII
9λ pool
2λ DNA (@.22 μg/λ)
5λ enzyme
16λ

Puc/SphI
9λ pool → 2λ DNA
5λ enzyme
16λ

not purifi
λ H III
9λ pool
2λ DNA
5λ enzyme
16λ

2:30 pm → 50' before stopping

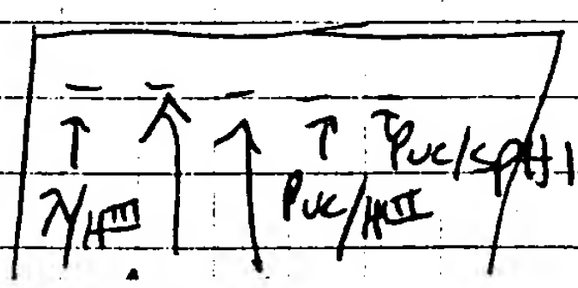
T=001.50 A=000138.6(±15%) B=000144.6(±15%) C=000166.6(±15%) R=1.050
T=000.50 A=000184.0(>20%) B=000192.0(>20%) C=000222.0(>20%) R=1.000

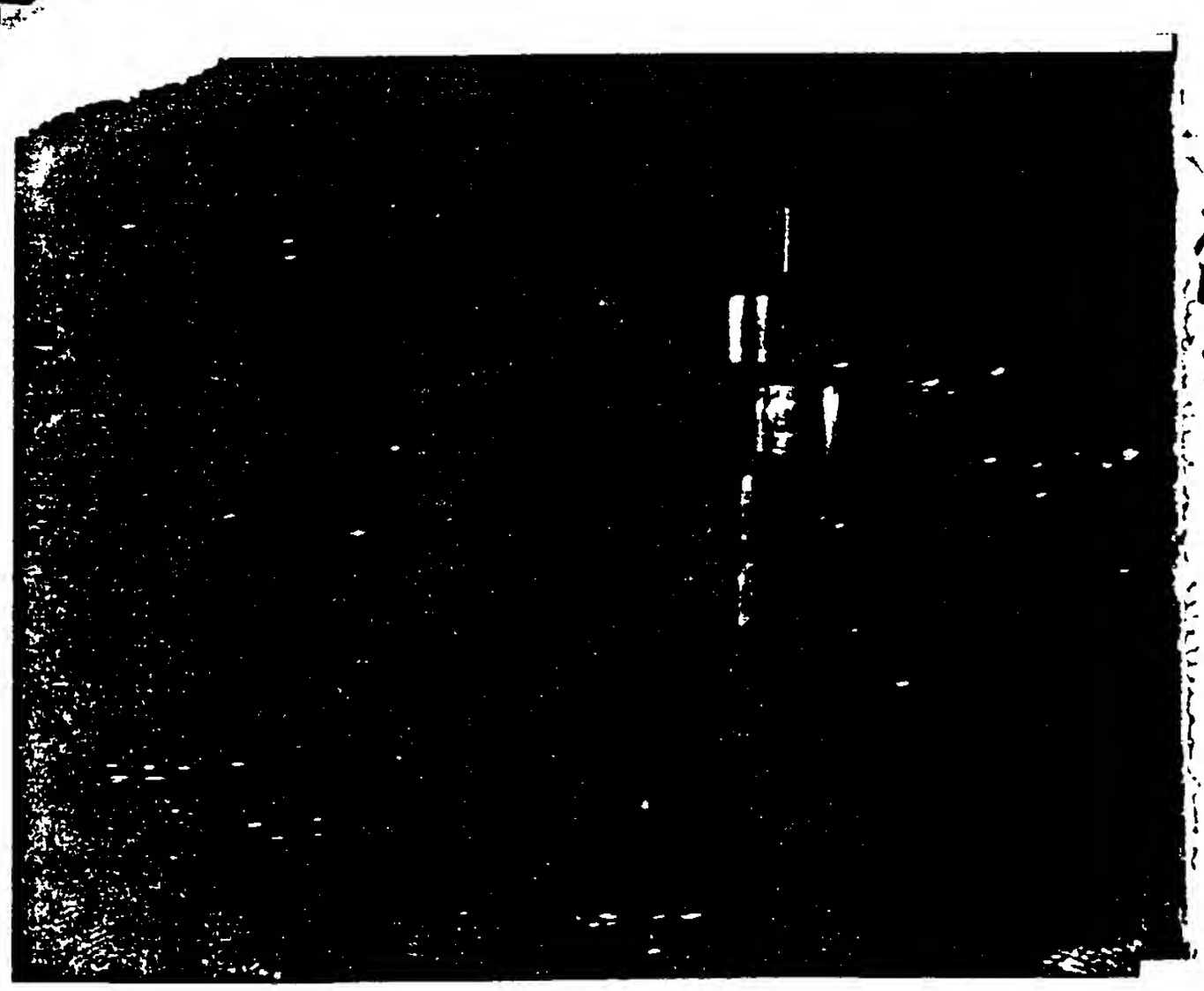
T=000.50 A=000162.0(>20%) B=000153.0(>20%) C=000202.0(>20%) R=0.825
T=000.50 A=000055.0(>20%) B=000076.0(>20%) C=000098.0(>20%) R=0.975

Lambda

The above rxn used poly(A)100 as a positive control (which worked ⇒ the problem is in the DNA and not the ~~reaction~~ terminal transferase)

Gel to find out if DNA present



λ/HIII
PUC
"PUC" → from unreliable source
PUC/HIII
PUC/SphI

⇒ DNA present

## TdT Assay

| pool | pertube | | final concentrati |
|---|---|---|---|
| 12.5λ | 2.5λ | 1M KTcacodylate pH7.0 | 100mM KCac |
| 2.5λ | 0.5λ | 50mM $CoCl_2$ | 1mM $CoCl_2$ |
| 50λ | 1λ | 1:100 dilution of 0.5Md.TT | 0.2mMd.TT |
| 2.5λ | 0.5λ | 5mM d.GTP | 1mM d.GTP |
| 250λ | 5λ | .087mM $^3$H d.GTP | .017mM d.GTP |
| | 1λ | .8 Mg/λ DNA (=.8mg) | 1.64×10$^{-7}$M |
| | 1λ | enzyme | (1) |
| | 13.5λ | $H_2O$ | |

d.GTP = 6,00
3' ends

## HindIII digest of PUC13

90λ PUC13 DNA (1μg/λ ⇒ 90μg)
22λ HindIII (=88 units)
12.4 10X Core buffer

124.4λ total

4:30pm

Digest was checked after 5hrs, but then allowed to go O/N

[redacted]

David's Energizer CBEC - 8 units (R)

30 Minutes

Poly(A) 1.0 T=000.50 A=000168.0(>20%) B=000[illegible]5.0(>20%) C=000202.0( 20%) R=1.000
Poly3 015 T=000.50 A=000144.0(>20%) B=000152.0(>20%) C=000[illegible]7.0(>20%) S=1.000

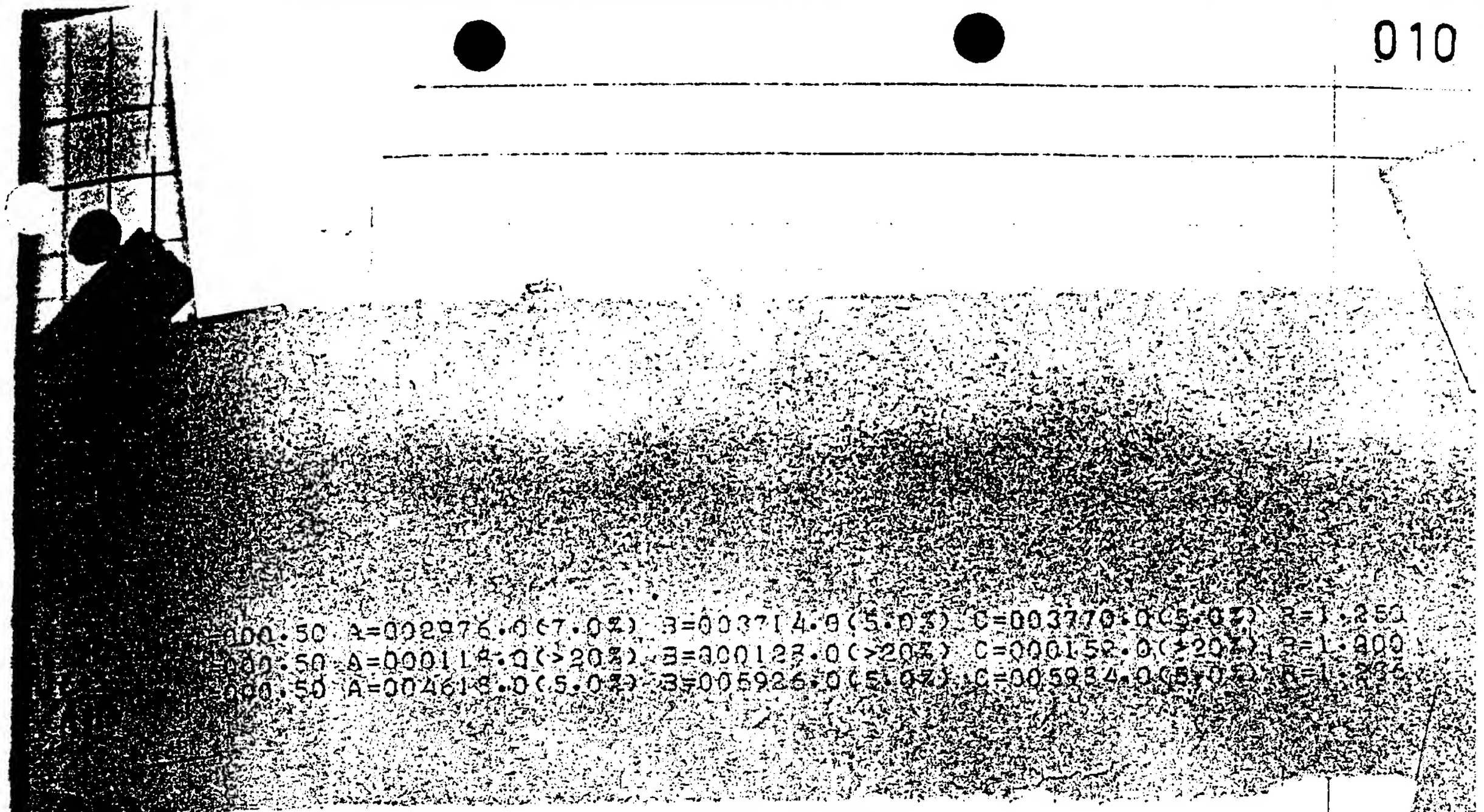
after 4 hours

1. Neg 1
2. Puc19 uncut
3. Puc19 uncut
4. Puc13/HindIII

# TdT Assay

| Poly(A)50 | Puc13/HindIII | Puc13/HindIII + Poly(A) | Control |
|---|---|---|---|
| 9λ pool | 9λ pool | 9λ pool | |
| 7λ poly(A) | 1 [redacted] DNA | 1 [redacted] DNA | |
| 1λ enzyme | 1λ enzyme | 7λ poly(A) | |
| 8λ $H_2O$ | 14.5λ $H_2O$ | 1λ enzyme | |
| 25λ | 25λ | 7.5λ $H_2O$ | |
| | | 25λ | |

↳ 30 min.




## #6's per 3' end of Puc13

Molarity of Puc13:

$$\frac{(1\times10^{-6}\ g)(1\ \text{mole})}{1.8\times10^{6}\ g} = 5.56\times10^{-13}\ \text{moles (in 1 } \lambda \text{ of DNA)}$$

~~$5.56\times10^{-13}\ \text{moles} = 2.22\times10^{-11}\ \text{mM}$~~
~~.025 ml~~

~~there are 2 3' ends per molecule $\Rightarrow (2)(2.22\times10^{-11}\ \text{mM}) = 4.44\times10^{-11}\ \text{mM}$~~

(cf. Wu who recommends ~~60 pmole/ml $= 6\times10^{-11}$ mM~~)

$$\frac{5.56\times10^{-13}\ \text{moles}}{25\times10^{-6}\ l} = 2.22\times10^{-8}\ M = 2.22\times10^{-5}\ \text{mM}$$

remember 2 3' ends/molecule $\Rightarrow 4.44\times10^{-5}$ mM (3'

Molarity of dGTP

$4.5\lambda \Rightarrow 25\lambda \Rightarrow \frac{4.5}{25} = .18$ dilution

$(.18)(50\text{mM}) = 9\text{mM}$

$$\frac{GTP}{PUC13} = \frac{9\,nM}{2.22\times10^{-5}\,nM} = 405{,}405\ G's/PUC13$$

max possible

$$\frac{GTP}{PUC13} = \frac{9\,nM}{4.44\times10^{-5}\,nM} = 202{,}703\ G/3'OH$$

fraction of counts incorporated

background

$$\frac{118-30}{(250\times4575)\,cpm} = \frac{88}{(250\times4575)} = 7.7\times10^{-5}$$

.17 g added pool = 4575 cpm in 25 g pool there are (250×4575 cpm)

actual incorporation:

$7.7\times10^{-5}\,(202{,}703) =$ **15 G/3' end**

#G's per 3' end on poly(A)

molarity of poly(A) ≠ (see also p. 4)

$$\frac{(2.5\times10^{-6}\,g)(7\times10^{-3}\,mol)}{(50)(347\,g/mole)} = 5.04\times10^{-11}\,mole$$

$$\frac{5.04\times10^{-11}\,mole}{25\times10^{-6}\,l} = 2.02\times10^{-6}\,M = 2.02\times10^{-3}\,mM$$

max possible incorp.

$$\frac{GTP}{PUC13} = \frac{9\,nM}{2.02\times10^{-3}\,mM} = 4{,}455\ G/3'OH$$

411

Notice that although $\frac{G}{3'end}$ is about the same for both Puc13/HIII and poly(A)50 the ratio of $\frac{GTP}{3'OH}$ is greater for Puc13 (⇒ equilibrium-mass action-effects should be stronger)

Since $\frac{GTP}{3'OH}$ for Puc13 is about 50X greater than the ratio for poly(A)50 and the amt of incorp is about the same, this suggests the Puc13 rxn is 50X less efficient than poly(A)50

(Obvious: The reason why there is relatively few counts depicted for Puc13/HIII is because the molarity of this is much less than that of the poly(A)50 as used in the same assay. To better differentiate background from incorporation in the Puc rxn, I should ↑ the amt of label in the pool-by 5cms, 10X (sounds good).)

## fraction actually incorporated

$$\frac{30000}{(250)(4575)} = .0026$$

actual incorp $(.0026)(4,455) =$ **12 6/3' end**

---

The molarity of $^3$HdGTP in stock

data from spec sheet →

$$\frac{.013 \times 10^{-3} g}{(.25 \times 10^{-3} L)(595.29 g/mol)} = 8.7 \times 10^{-5} M = 87 \times$$

$$= .087 mM$$

| poly(A)$_{50}$ | pUC13/H$^{III}$ | Control |
|---|---|---|
| 9λ pool | 9λ pool | 9λ pool |
| 7λ poly(A) | 1λ DNA | 2λ DNA (poly(A)$_{50}$) |
| 1λ enzyme | 1λ enzyme | 1λ enzyme |
| 8λ $H_2O$ | 14λ $H_2O$ | 8λ $H_2O$ |
| 25λ total | 25λ total | 20λ total |

put in at 37° at 3:30pm
↳ allowed to go for 18 hours

[redacted]

poly(A)$_{50}$ →

[redacted]

pUC13/H$^{III}$ →

016 T=000.50 A=000178.0(>20%) B=000176.0(>20%) C=000300.0(>20%) R=1.000

control (poly(A)DNA) →

017 T=000.50 A=000052.0(>20%) B=000070.0(>20%) C=000104.0(>20%) R=1.500

18 HOUR ASSAY

[redacted]

$PuCl_3/H^{III} \Rightarrow$ 226 l/3 end

$payd(A)_{SO} \Rightarrow$ 176 l/3 end

dGTP, $Na^+$ salt ~~507.20~~ 609 g/mole

Pt ~~dimers~~ new kits .025g

1ml 1M soution

TdT Assay

This assay uses 1/10 and 1/100 the molar concentration of PUC13/HIII as the previous series. This effectively ↑ the relative d6TP/3'OH ratio by a factor of 10. Additionally the label has been increased by 14-fold. (There should be more incorporation per 3' end, but there are fewer 3' ends, so this increase is not expected to appreciably alter the counts, relative to background.)

1. $\frac{1}{10}$ the previous concentration of DNA ⇒ $2.03 \times 10^6$ excess of d6TP over 3'

| | | | |
|---|---|---|---|
| ✓ | 1λ | of a 1:10 dilution of 1μg/1λ PUC13/HIII DNA | .1 μg = $4.44 \times 10^{-6}$ mM |
| | 1λ | TdT | 3 units |
| ✓ | 5λ | 50mM d6TP | 10mM |
| | 14λ | $^3$H d6TP | .05mM |
| ✓ Pool = | 2.5λ | 1M KCacodylate | 100mM |
| ↳ 4λ | 0.5λ | 5mM CoCl$_2$ | 1mM |
| | 1λ | 1:100 dilution of 0.5M diTT | 02mM diTT |
| | 25λ | total | |

2. ~~12 of a 1:100 dilution of~~ 2.03NO$^7$

$\frac{1}{100}$ the previous concentration of DNA ⇒ ~~[illegible]~~ excess of d6TP over 3'ends

1λ of a 1:100 dilution of 1μg/1λ PUC13/HIII DNA — .01 μg = $4.44 \times 10^{-7}$ mM

~~Above~~ the rest is the same as the above assay

25λ

3. As before, 1λ of 1μg/1λ PUC13/HIII DNA
The rest is the same as above

4. Control, everything, but no DNA or enzyme — $2.03 \times 10^5$ Co / 3'end

1 λ of the 23λ total control rxn was spotted for use as a "pat spot"

Poo ⊖ ← signifies no dGTP
(enough for 100 25λ assays)

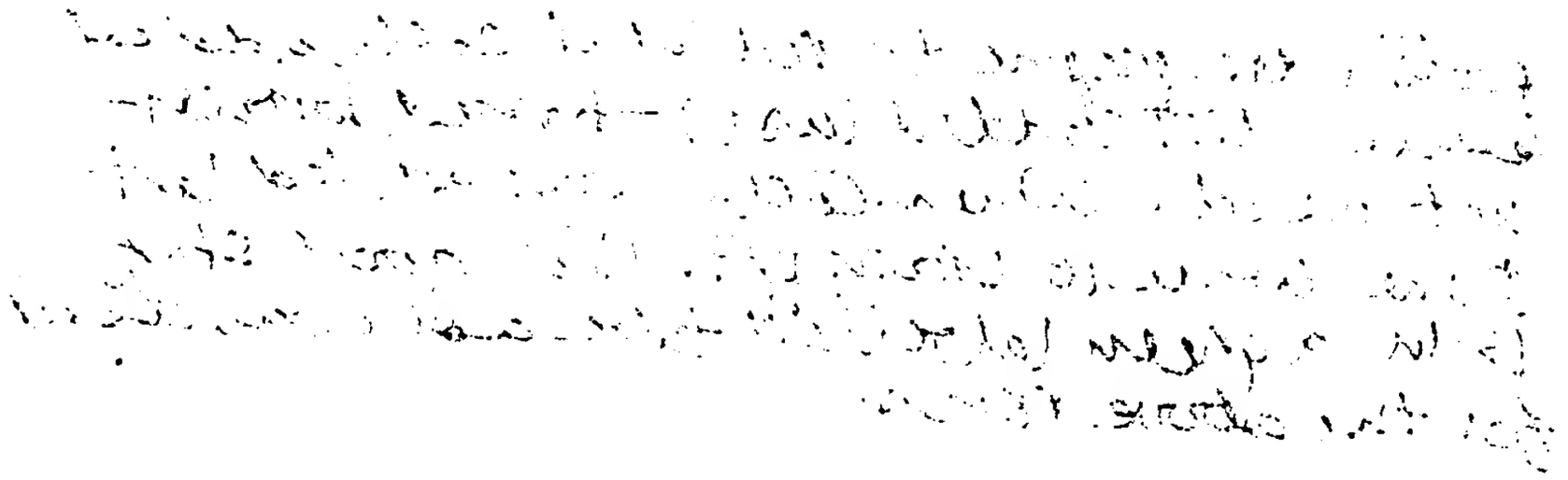
250λ cacodylate
50λ $CoCl_2$
99λ $H_2O$
1λ undiluted 0.5M diTT

2 pots were prepared - red label $CoCl_2$ added before diTT added (last) - turned brown - not used. When $CoCl_2$ was added last there was no brown ppt. This good stuff is in a green labelled tube and was used for the above rxns.

```
1 T=000.50 A=000670.0( 15%) B=000732.0( 15%) C=000754.0( 15%) R=1.090
2 T=000.50 A=000334.0( 20%) B=000456.0( 15%) C=000484.0( 15%) R=1.157
3 T=000.50 A=000400.0( 15%) B=000490.0( 15%) C=000512.0( 15%) R=1.200

1 T=000.50 A=000460.0( 15%) B=000559.0( 15%) C=000570.0( 15%) R=1.173
2 T=000.50 A=211804.0(0.7%) B=248909.0(0.7%) C=250060.0(0.7%) R=1.174
```

Perhaps the excess etOH inhibited the rxn, or there may also be substrate level inhibition



Idea: put in *dTTP as trace in dGTP talics. What will this tell?

~30 minutes

TdT assay

1. 1λ of DNA - puc[13] (H+III cl/µg(λ) — 1µg
   1λ TdT — 3 units
   1λ α-32P dTTP (Evaporated and redelted)
   4λ Pool — std
   18λ 50mM dTTP — 36µM dTTP
   25λ

2. Same as above but 1λ of .1µg/λ puc[13] H+III — .1µg

3. as above, but 1λ of .01µg/λ puc[13] H+III — .01µg

4. control
   no DNA
   no TdT
   ---
   23λ total
   ~~spot on gel~~
   dilute 1λ 1:10 then spot

5≡5. Check to see that such massive amts of dNTP's are all causing substrate-level inhibition

   1λ of 1µg/λ pc DNA
   1λ TdT
   1λ α-32P dTTP
   4λ Pool-1
   1λ ~~50mM~~ of 1:4 dilution of 50mM dTTP ← .5mM
   ~~12λ~~
   17λ H2O
   ---
   25λ total

Pool Spot: 1λ of 1:10 dilution ← from control (23λ tube)

PROG 6 USER 1

SR CH 12 1 TIMES
CR 1 TIMES
SCR =OFF
AQC =OFF
SF =ON
CALC= 1
PST = 10.00 MIN
CH 1 2.00 2 SIGMA %
.0 LSR
.0 BKG
.00 2 SIGMA B
0 LL
397 UL
CH 2 2.00 2 SIGMA %
.0 LSR
.0 BKG
.00 2 SIGMA B
655 LL
940 UL

| POS | CH 1 | 2S% | CH 2 | 2S% | TIME |
|---|---|---|---|---|---|
| 8 | 156.4 | 5.0 | 5622.1 | .8 | 10.00 |
| 9 | 22.9 | 13.2 | 472.8 | 2.9 | 10.00 |
| 10 | 22.7 | 13.2 | 316.9 | 3.5 | 10.00 |
| 11 | 3624.6 | 1.9 | 202151.8 | .2 | 2.76 |
| 12 | 18.4 | 14.7 | 235.9 | 4.1 | 10.00 |
| 13 | 281.3 | 3.7 | 9136.4 | .6 | 10.00 |

Calculations for above experiment

1. 1 µg/1 DNA

$\frac{GTP}{PUC13} = \frac{10\ mM}{4.44 \times 10^{-6}\ mM} = 2.25 \times 10^{5}$ ← max possible incorporation

fraction of counts incorporated

$\frac{5622.1 - 235.9}{(9136.4)(1.087 \times 10^{25})} = \frac{5386.2}{2,482,816.7} = .00217$

(9136.4) ← diluted 1:10

$1.087 \times 10^{25}$ ← x25 to get total possible counts

$(\frac{1}{23})(25\lambda) = 1.087\lambda$

↑ 1λ from a total volume of 23λ is equal to 1.087 from a final vol of 25λ

actual incorporation $(.00217)(2.25 \times 10^{5}) = 488$ T/3'end

2. 1 µg/1 DNA

$\frac{GTP}{PUC13} = \frac{10\ mM}{4,44 \times 10^{-6}\ mM} = 2,25 \times 10^{6}$ ← max possible incorp

fraction of counts incorporated

$\frac{478.2 - 235.9 = 242.3}{2,482,816.7} = 9.76 \times 10^{-5}$

actual incorp → $(9.76 \times 10^{-5})(2,25 \times 10^{6}) = 220$ T/3'end

~~Bill~~ 1 of .01 µg/λ PuCl3/H

max possible incorp → $2.25 \times 10^7$

fraction of counts incorp → $\frac{3169 - 2359}{2,482,867} = \frac{\approx 81}{3,26 \times 10^5}$

actual incorp → $(2.25 \times 10^7)(3.26 \times 10^{-5}) = 734$

~~Will~~

S.E.S 1 λ of 1 µg/λ PuCl3/H - diff conc of dTTP

• molarity of dTTP $(\frac{1}{100})(50mM) = .5mM$

max possible incorp = dTTP / 3'OH = $\frac{.5mM}{4.44 \times 10^5 mM} = 1.13 \times 10^4$

fraction of counts incorp → $\frac{202,151.8 - 235.9}{2,482,816.7} = \frac{\approx 201,915.9}{.0813}$

actual incorp → $(.0813)(1.13 \times 10^4) = 919$ T/3' end

# TdT Assay

1. 4λ pool — std'd
   1λ pUC13/HIII @ 1μg/1λ — 1μg
   5λ $^3$H dGTP-evap & reeluted
   1λ TdT — ~3 units
   5λ 1:10 dilution of — 1mM
   50mM dGTP
   9λ $H_2O$
   25λ

2. 4λ pool
   1λ pUC13/HIII
   5λ $^3$H dGTP-evap & reeluted
   1λ TdT
   10λ 1:10 dilution of 50mM dGTP — 2mM dGTP
   4λ $H_2O$
   25λ

3. Same as above, but
   2.5λ 1:10 dil. of 50mM dGTP — 0.5mM dGTP
   11.5λ $H_2O$
   25λ

4. 1λ 1:10 dil. of 50mM dGTP
   13λ $H_2O$ — 0.2mM dGTP
   25λ

200λ of $^3$H dGTP was vac dryed & reeluted in 40λ TE (⇒ 5X concentration)

~~(Φλ~~ $H_2O$

~~a~~ 1λ PUC 13/11$^{III}$ @ 1µg/1λ — 1µg
1λ TdT — ~3 units
1λ $\alpha$-$^{32}$P dTTP
~~4~~ 1λ Pool$^{-1}$
2λ @ a 1:4 dilution @ 50mM dTTP ⇒ 1mM dTTP
run for: 25λ total
10Min
30Min ⇒ 4 separate runs
1hour
2hour

↳ NO DNA ~~control~~ TdT
+ Control (23λ total)
+ pool-spot 1λ from control
+ "Save" 30Min for the sequencing experiment

On "Save" - don't stop w/ acid wash - stop by chilling - it will later be phenol extracted & then cut & run out on gel

10Min 005 T=000:50 A=258594.0(0.7%) B=207376.0(0.7%) C=303772.0(0.7%) R=1.151
30Min 006 T=000:50 A=273180.0(0.7%) B=275920.0(0.5%) C=332902.0(0.5%) R=1.193
60min 007 T=000:50 A=300768.0(0.7%) B=253172.0(0.5%) C=365600.0(0.5%) R=1.190
2hr 008 T=000:50 A=195122.0(0.7%) B=219502.0(0.7%) C=223372.0(0.7%) R=1.125
009 T=000:50 A=000564.0(.15%) B=000768.0(.15%) C=000856.0(.10%) R=1.151
Control 010 T=000:50 A=151398.0(1.0%) B=200524.0(0.7%) C=207264.0(0.7%) S=1.151
Pool 1λ undil. from orig. 23λ control

Remember, the calculated values are per 3' end

30 minutes

$$\frac{dTTP}{@3'OH} = \frac{1mM}{4.44NO-5mM} = 2.25X10^4$$

$$\frac{273,180-664}{(151,398)(\frac{25}{23})(25)} = \frac{272,516}{4,114,076.1} = .0662$$

$$(.0662)(225X10^4) = 1,490\ T/3'end$$

10 minutes

$$\left(\frac{258,694-664}{4,114,076.1}\right)(2.25X10^4) = 1,411\ T/3'end$$

1 hour

$$\left(\frac{300,768-664}{4,114,076.1}\right)(2.25X10^4) = 1,641\ T/3'end$$

2 hours

$$\left(\frac{195,122-664}{4,114,076.1}\right)(2.25X10^4) = 1064\ T/3'end$$

check to see if ligation works

idea: in plastic bag put 5 pcs 1:10 dil of all 4 bases

- precipitate the sample for the gel
- try all 4 in labelling rxns

## TdT Assay w/ all 4 bases present

| | | |
|---|---|---|
| 1. 1λ | PUC13/HIII DNA @ 1μg/λ | 1μg |
| 1λ | TdT @ ~30U/λ | 30U |
| 4λ | Pool⁻ | St'd |
| 1.3λ | 1:10 dil of 50mM d6TP (=5mM) | 0.25mM d6TP |
| 1.3λ | 1:10 dil of 50mM dCTP (=5mM) | 0.25mM dCTP |
| 1.3λ | 1:10 dil of 50mM dATP (=5mM) | 0.25mM dATP |
| 1.3λ | 1:10 dil of 50mM dTTP (=5mM) | 0.25mM dTTP |
| 1λ | α-32P dTTP (evap & reeluted) | 32P-T-label |
| 5λ | 3H d6TP (evap & reeluted) | 3H-G-label |
| 7.8λ | $H_2O$ | |
| 25λ | total | |

| | | |
|---|---|---|
| 2. 1λ | PUC13/HIII DNA @ 1μg/λ | 1μg |
| 1λ | TdT @ ~3U/λ | 3U |
| 4λ | Pool⁻ | St'd |
| 1.3λ | 1:10 dil of 50mM d6TP | 0.25mM d6TP |
| 1.3λ | " dCTP | " dCTP |
| 1.3λ | " dATP | " dATP |
| 1.3λ | " dTTP | " dTTP |
| 1λ | α-32P dCTP | 32P-C-label |
| 5λ | 3H dATP (evap & reeluted) | 3H-A-label |
| 7.8λ | $H_2O$ | |
| 25λ | total | |

C1 ≡ 3. Control - for rxn #1

| | |
|---|---|
| 4λ | Pool⁻ |
| 1.3λ | 1:10 dil of 50mM d6TP |
| 1.3λ | " dCTP |
| 1.3λ | " dATP |
| 1.3λ | " dTTP |
| 1λ | α-32P dTTP |
| 5λ | 3H d6TP |
| 9.8λ | $H_2O$ |

C2 ≡ 4. Control - for rxn #2

| | |
|---|---|
| 4λ | Pool⁻ |
| 1.3λ | d6TP |
| 1.3λ | dCTP |
| 1.3λ | dATP |
| 1.3λ | dTTP |
| 1λ | α-32P dCTP |
| 5λ | 3H d6TP |
| 9.8λ | $H_2O$ |

From each of the controls spot 17 as the "pool"

1, 2, C1, C2, P1, P2

→ 40 min

INCORP of G

$$\frac{\text{Max possible}}{\text{G incorp}} \rightarrow \frac{\text{dGTP}}{3'\text{end}} = \frac{0.25\text{mM}}{4.44\times10^{-5}\text{mM}} = 5{,}631$$

$$\frac{\text{fraction of counts}}{\text{incorp}} \rightarrow \frac{136{,}890 - 570}{(122{,}262)(25)} = .0446$$

$$\frac{\text{actual}}{\text{incap}} \rightarrow (.0446)(5{,}631) = 251$$

INCORP of A

$$\frac{\text{max possible}}{\text{A incorp}} \rightarrow \frac{0.25\text{uM}}{4.44\times10^{-5}\mu\text{M}} = 5{,}631$$

$$\frac{139{,}538 - 584}{(131{,}524)(25)}(5{,}631) = 238$$

incorp of C

$$\frac{201,442 - 926}{(412,726)(25)}(5,631) = 109$$

incorp of T

$$\frac{47,096 - 266}{(113,821)(25)}(5,631) = 93$$

| | base/blend | % |
|---|---|---|
| A | 238 | 34.4% |
| C | 109 | 15.8% |
| G | 251 | 36.3% |
| T | 93 | 13.5% |
| | 691 | 100% |

$\Rightarrow G > A > C > T$

[redacted]

A: $1/2\ {}^{3}H$ B: $^{32}P$ [redacted] C: $^{3}H + {}^{14}C + {}^{32}P$

[redacted]

Run over again, exactly the same, w/ the exception of #2, which will just be stopped & run out on a gel
↑ two #2's

40 minutes, as before

[redacted]

[redacted]

A T=001.00 A=000959.0(2.0%) B=000157.8( 1.5%) C=001560.0(7.0%) R=0.368
[redacted] T=001.00 A=000549.0( 10%) B=000516.5( 10%) C=001333.8(7.0%) R=0.942

[redacted] T=001.00 A=281265.0(0.5%) B=059562.0(1.0%) C=432974.0(0.3%) R=0.347
[redacted] T=001.00 A=174665.0(0.5%) B=279508.0(0.5%) C=551349.0(0.3%) R=1.600
[redacted] T=001.00 A=177395.0(0.5%) B=113914.0(0.7%) C=324891.0(0.5%) R=0.642
[redacted] T=001.00 A=183277.0(0.5%) B=324967.0(0.5%) C=593820.0(0.3%) S=1.773

same channels as above

[redacted]

⇓

6 → $\frac{281,265-959}{(177,395)(25)}(5,631) = 356$

A → $\frac{174,665-549}{(183,277)(25)}(5,631) = 214$

C → $\frac{279,508-516}{(324,967)(25)}(5,631) = 193$

T → $\frac{69,662-357}{(113914)(25)}(5,631) = 137$

| | | % |
|---|---|---|
| G | 356 | 40% |
| A | 214 | 24% |
| C | 193 | 21% |
| T | 137 | 15% |
| Total | 900 | |

↳ G > A > C > T

---

The saved portion from the pellet tubing (@ 15 pellet) was frozen after the rxn was completed (30 min). Then, together w/ the "saved portion" from the experiment immediately above, the two were phenol extracted & EtOH ppt'd

↓ re-eluted

each, ~~12.5λ~~ ~~600~~ $H_2O$ ← ~1 µg in rxn mix

1λ SphI = 5 units

1.5λ 10X low salt buffer

15λ total

37°C @ 8:40 am

↳ 2 hours, 20 mins long

↳ both of these samples are loaded on a sequencing gel run by Clark

-Calculations of Stop codon appearance based on base composition

Term. Codons: UAA UGA ⇒ TAA TGA
UAG TAG

Using data from the first experiment w/ all 4 n.t.'s present

$(T)(A)(A) + (T)(A)(G) + (T)(G)(A)$ where T = prob of T = .135
A = etc. = .344
C = .158
G = .363

~~$(.135)(.344)^2 + (.135)(.344)(.363) + (.135)$~~

$= (T)(A^2) + 2(T)(A)(G)$

$= (.135)(.344)^2 + (2)(.135)(.363)(.344)$
$= .04969$

| # of residues | Fraction eliminated | | |
|---|---|---|---|
| 1 | .95 | .983 | .983 .98 |
| 10 | .60 | .843 | .843 .82 |
| 30 | .22 | .60 | .60 .56 |
| 50 | .078 | .422 | .422 .38 |
| 75 | .022 | .279 | .279 .22 |
| 100 | .006 | .182 | .182 .14 |
| 150 | .00047 | .078 | .078 .05 |
| 200 | .000037 | .033 | .033 .02 |
| 300 | .0000002 | .006 | .006 .03 |

A = .15
T = .15
C = .30
G = .35

A = .15
T = .15
C = ~~.30~~ .40
G = ~~.35~~ .30

$(.15)(.15)^2 + 2(.15)(.15)(.35) =$

Anticipated N.T. concentrations

A: ~~(.34/.15)(.25mM)~~ =

desired → (.15/.34)(.25mM) = .11mM
actual →

T: (.15/.135)(.25mM) = .28mM

C: (.40/.158)(.25mM) = .63mM

G: (.30/.363)(.25mM) = ~~.165~~ .21mM

1.23mM total [N.T.]

these values are calculated per mg of DNA

# TdT Assay (Try out previously calculated concentrations & check the new PL terminal transferase

1. 1λ pUC13/HIII DNA @ 1μg/λ — 1 μg
   1λ lab TdT (~30 units/λ) — 30 units
   4λ pool — Std'd Concentration
   2.2λ 1:40 dil of 50mM dATP (=1.25mM) — .11mM dATP
   1.4λ 1:10 dil of 50mM dTTP (=5mM) — .28mM dTTP
   3.2λ 1:10 dil of 50mM dCTP (=5mM) — .63mM dCTP
   1.1λ 1:10 dil of 50mM d(G)TP (=5mM) — .21mM d(G)TP
   1λ α-32P dTTP
   3λ 3H d(G)TP
   7.1λ $H_2O$

2. same as above except
   1λ α-32P dCTP
   3λ 3H dATP
   25λ

3. Control for rxn 1
   as above# but no DNA or TdT
   ↳ 9.1λ $H_2O$
   25λ

4. Control for rxn 2
   as #2 but no DNA or TdT
   ↳ 9.1λ $H_2O$
   25λ

---

PL TdT
repeat above: ~~all identical~~ use same control;
1 & 2 use just 0.5λ TdT
and compensate w/ 7.6λ $H_2O$
↳ 1' & 2' thaw labelled

Take 2λ from each control and spot to use as a "pool" spot#

~~1:45~~ → 40 minutes

Stock HdATP was diluted, so that there'd be enough for this experiment

C(124 T=000.50 A=00292.0(20%) B=00284.0(20%) C=00742.0(15%) R=1.000
C(125 T=000.50 A=00166.0(>20%) B=00388.0(10%) C=01338.0(10%) R=1.500
C(126 T=000.50 A=22154.0(0.7%) B=040312.0(1.5%) C=350902.0(0.5%) R=0.271
C(127 T=000.50 A=01394.0(3.0%) B=494348.0(0.5%) C=593344.0(0.5%) R=4.271
C(128 T=000.50 A=03137.0(2.0%) B=00[illegible]0.0(3.0%) C=04[illegible]2.0(1.5%) R=0.283
C(129 T=000.50 A=00247.0(7.0%) B=072900.0(1.5%) C=03370[illegible].0(1.0%) R=0.440
C(130 T=000.50 A=23014.0(0.7%) B=273562.0(0.7%) C=34450.0(0.5%) R=1.202
C(131 T=000.50 A=02[illegible]54.0(2.0%) B=69174.0(0.5%) C=94[illegible]00.0(0.2%) R=1.202

Same channels as before

→ A= 12C 17 32
B=
C=

Lab Talk

[A] $\frac{dATP}{3'} = \frac{.111mM}{4.44\times10^{-5}mM} = 2,478$

$\frac{13,948-166}{29,454(12.5)}(2,478) = 93$

[G] $\frac{dGTP}{3'} = \frac{.21uM}{4.44\times10^{-5}uM} \simeq 4,730$

$\frac{221,554-292}{230,414(12.5)}(4,730) = 363$

[C] $\frac{dCTP}{3'} = \frac{.63mM}{4.44\times10^{-5}uM} \simeq 14,189$

$\frac{494,348-888}{199,174(12.5)}(14,189) = 801$

T $\frac{dTTP}{31} = \frac{.28mM}{4.44 \times 10^{-5} mM} = 6,306$

$\frac{60,312 - 284}{278,562 (42.5)} (6,306) = 109$

P-L TdT

A $\frac{2,472 - 166}{29,454 (12.5)} (2,478) = 16$

G $\frac{31,372 - 292}{230,414 (12.5)} (4,730) = 51$

C $\frac{22,920 - 888}{699,174 (12.5)} (14,189) = 117$

T $\frac{8,898 - 284}{278,562 (12.5)} (6,306) = 16$

| | P-L # | P-L % | Lab Prep # | Lab Prep % | Calculat Goal |
|---|---|---|---|---|---|
| A | 16 | 8% | 93 | 7% | 15 |
| C | 117 | 59% | 801 | 59% | 40 |
| T | 16 | 8% | 109 | 8% | 15 |
| G | 51 | 26% | 363 | 27% | 30 |
| total | 200 | | 1366 | | |

appearance of term. codons for above base ratios

$(T)(A^9) + 2(T)(A)(6)$

$(.08)(.08)^2 + (2)(.08)(.08)(.26)$

| residues | % unstopped |
|---|---|
| 1 | .9962 |
| 50 | .825 |
| 100 | .68 |
| 150 | .56 |
| 200 | .46 |
| 250 | .38 |
| 300 | .32 |
| 350 | .26 |
| 400 | .21 |
| 500 | .15 |
| 600 | .10 |
| 700 | .07 |
| 1000 | .02 |

← 455 ⇒ 17% still intermediate

(correlates curve with syndrome)

⇒ look to see how aa distribution ratio is affected

A New calculating anticipated [dNTP] to get the desired A=.15 (chosen on p.19 quote)
T=.15
C=.40
G=.30

A: $\frac{.15}{.06}(.11) = .24\,mM$ ← though I know that this conc. ratio before gave me too much

T: $\frac{.15}{.06}(.28) = .53\,mM$

C: $\frac{.40}{.159}(.63) = 1.42\,mM$

G: $\frac{.30}{.27}(.21) = \frac{.23\,nM}{1.42\,nM}$

I think that the problem w/ this sort of calculation is that one is dependent upon the other so that these should be "normalized" w/respect to each other in any calculation

target

desired goal: A = .05
C = .25
G = .25
T = 45

This is one way to calculate expected concentrations

& also some insight to run out on a gel

| | fraction in product | | major incorp fraction | rxn fraction = product fraction = M |
|---|---|---|---|---|
| last experiment → | A = .07 | .11 mM | .082 | .082/.07 = 1.17 |
| | C = 0.59 | .63 mM | .47 | .47/.59 = .80 |
| | T = .08 | .28 mM | .209 | .209/.08 = 2.61 |
| | G = .27 | .21 mM | .157 | .157/.27 = .581 |
| | | 1.34 mM | | |

| | desired product fractions | rxn fraction (estim.) = M (desired product fraction) |
|---|---|---|
| A | .05 | .0684 |
| C | .25 | .20 |
| G | .25 | .653 |
| T | .45 | .261 |
| | | 1.18 |

⇓

normalize w/ respect to 1

| | |
|---|---|
| A | .0684/1.18 = .058 |
| C | .20/1.18 = .17 |
| G | .653/1.18 = .55 |
| T | .261/1.18 = .22 |
| | .998 |

1. 1λ Pst 13 IHIII DNA @ 1μg/λ
   1λ TdT
   4λ Pool
   1.2λ 1:40 dil of 50mM dATP (=1.25mM)
   3.4λ 1:40 dil of 50mM dCTP (=1.25mM)
   2.8λ 1:10 dil of 50mM dGTP (=5mM)
   1.1λ 1:10 dil of 50mM dTTP (=5mM)
   1λ $\alpha$-32P dCTP
   3λ 3H dGTP
   6.5λ $H_2O$
   25λ

1μg
~3units
St'd concentrations
~~.058mM~~ dNTP ~~(.06)~~ .06
.17mM dCTP
~~.55~~ .55mM dGTP
.22mM dTTP

2. Same as above but
   1λ $\alpha$-32P dCTP
   3λ 3H dATP

Save ≡ 1

For ~~controls~~ pool before adding TdT to the 3 rxn mixtures draw out 2λ and spot to count-replace w/ 2λ of $H_2O$. So, there'll be a C1
C1' (≡ Save)
C2

Another way to calculate out expected concentrations

Via ratio of dNTP/3'

| | | [dNTP]/[B] | | anticipated [dNTP] |
|---|---|---|---|---|
| A=.07 | .11nM | 2,478 | .05/.07(2,478) = 1,770 | .079nM |
| C=.59 | .63nM | 14,189 | .25/.59(14,189) = 6,012 | .27nM |
| T=.08 | .28nM | 6,306 | .25/.08(6,306) = 19,706 | .87nM |
| G=.27 | .21nM | 4,730 | .45/.27(4,730) = 7,883 | .35nM |

↑ total tail length
1366

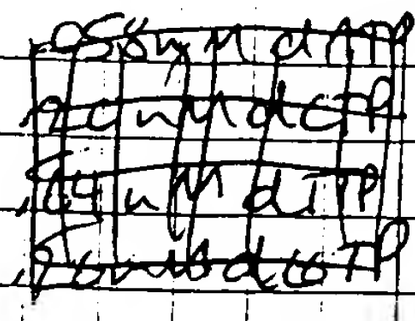
total tail length of 1,000 would be better, so multiply all [dNTP]'s by 1,000/1366 (=.732)

| | [dNTP] |
|---|---|
| A | .058nM |
| C | .20nM |
| T | .64nM |
| G | .26nM |
| | 1.16nM |

1, 3, 4 Pvc 13 III DNA @ 1 µg 1λ

1λ TdT
4λ pool
1.2λ 1:40 dilution 5mM dATP
1λ 1:10 dilution 50mM dCTP
3.2λ 1:10 dilution 5mM TTP
1.3λ 1:10 dilution 50mM dGTP
1λ α-32P dATP
3λ 3H dGTP
8.2λ $H_2O$
25λ total

1 µg
~3 units




2 Same as above, but
1λ α-32 dCTP
3λ 3H dATP

3 Same = 2' (same as 2)

For foot spot: Just prior to adding the TdT withdraw 2λ of rxn and spot. Then to restore original concentration add 2λ of $H_2O$. Then add TdT. Incubate same everything well mixed)

||

3 foot spots
P1
P2' (= same)
P2

40 minutes

The above isn't such a good way to do this because there is no background level produced from the neg. cont
(I also forgot to save "save" & acid ppt it by mistak

001 T=000.50 A=228682.0(0.7%) B=099116.0(1.0%) C=398834.0(0.5%) R=0.423
002 T=000.50 A=010042.0(3.0%) B=650738.0(0.5%) C=702653.0(0.5%) R=5.080
003 T=000.50 A=005706.0(5.0%) B=442938.0(0.5%) C=506555.0(0.5%) R=9.071
004 T=000.50 A=230364.0(0.7%) B=287778.0(0.7%) C=675748.0(0.5%) R=1.249
005 T=000.50 A=015394.0(3.0%) B=842870.0(0.3%) C=141430.0(0.3%) R=5.447
006 T=000.50 A=009122.0(3.0%) B=681974.0(0.5%) C=864122.0(0.3%) R=5.075

[A] $\frac{dNP}{3'} = \frac{.06mM}{4.44\times10^{-5}mM} = 1351$

[B] $\frac{dNP}{3'} = \frac{.26}{4.44\times10^{-5}} = 5,85$

$2 \Rightarrow \frac{10412}{15,344(12.5)}(1351) = 71$

$\frac{228,682}{230,364(12.5)}(5,856)$

$\approx 465$

$2' \Rightarrow \frac{5,706}{(9122)(12.5)}(1351) = 69$

[C] $\frac{dNP}{3'} = \frac{.20}{4.44\times10^{-5}} = 4,505$

[D] $\frac{dNP}{3'} = \frac{.64}{4.44\times10^{-5}} = 14,41$

$\frac{99,116}{287,778(12.5)}(14,414)$

$= 397$

$2 \Rightarrow \frac{650738}{442,870(12.5)}(4,505) = 278$

$2' \Rightarrow \frac{442,938}{681,974(12.5)}(4,505) = 224$

| | # | % | counted |
|---|---|---|---|
| A | 70 | 5.90% | 5 |
| C | 251 | 21.2% | 25 |
| G | 468 | 39.5% | 25 |
| T | 397 | 33.5% | 45 |
| | 1186 | 100.1% | |

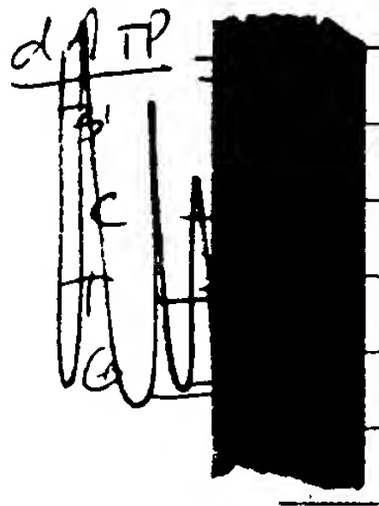
[redacted]

A = .059 C = .211 G = .395 T = .335

[redacted]

```
term =  1.678049E-02
ala =   .083345
arg =   9.392546E-02
asn =   1.900626E-03
asp =   1.272453E-02
cys =   7.224946E-02
gln =   5.651846E-03
glu =   1.058047E-02
gly =   .156025
his =   6.797154E-03
ile =   1.195783E-02
leu =   .1216352
lys =   1.580374E-03
met =   7.807176E-03
phe =   6.127485E-02
pro =   .044521
ser =   8.340953E-02
thr =   .012449
trp =   5.226838E-02
tyr =   1.079169E-02
val =   .132325
```

[redacted]



## TdT Tailing

Goal: In the large scale tailing it would be more convenient to use more concentrated DNA & dNTP, while maintaining the same dNTP/3'OH ratio. This has the advantage of a smaller total rxn volume and the requirement for less TdT. Therefore, this TdT assay will use greater concentrations.

Also, it would be nice to compare tailed vs. untailed plasmid on an agarose gel. Some of the DNA from this rxn will be run on a gel

### Procedure

1. 2 λ PuC13/III$^{m}$ @ 1 μg/λ
   4 λ gel
   1 λ of 1:5 dil of TdT
   2.3 λ 1:40 dil of 5 mM dATP
   2 λ 1:10 dil of 5 mM dCTP
   6.4 λ 1:10 dil of 5 mM dTTP
   2.6 λ 1:10 dil of 5 mM dGTP
   1 λ $^{\alpha\text{-}32}$P dTTP
   3.7 λ $^{3}$H dGTP
   25 λ

   2 μg DNA
   Std
   (This makes for 1/10 the amt used in PM in assay)
   .115 mM dATP
   .4 mM dCTP
   1.28 mM dTTP
   .52 mM dGTP

2. Same as above but
   1 λ $^{\alpha\text{-}32}$P dCTP
   3.7 λ $^{3}$H dATP
   25 λ total

1:40 pm

Before adding the TdT, withdraw 2λ and spot on disk to count. (Do not replace with 2λ $H_2O$ – if the mixture is homogenous before withdrawl then the concentrations will remain the same; the total volume will just decline slightly.)

After the rxn goes for its designated length withdraw 8λ from each rxn mixture ($\frac{8}{25}$ (2μg) = .64μg DNA) to run out on gel. Then stop rxn and count & ppt as before.

Remember, in the calculations, only $\frac{23-8}{25}$ (2μg) = 1.2μg will remain

[redacted]

1 P1 P2

[redacted]

Remainders only 237-87=150 was counted

A  dNTP / 3'OH = .115 nM / 8.85x10^-5 nM = 1295

18086 / 293,886 (7.5) (1295) = 11

C  dNTP / 3'OH = .4 nM / 8.85x10^-5 nM = 4505

45002 / 879,946 (7.5) (4505) = 31

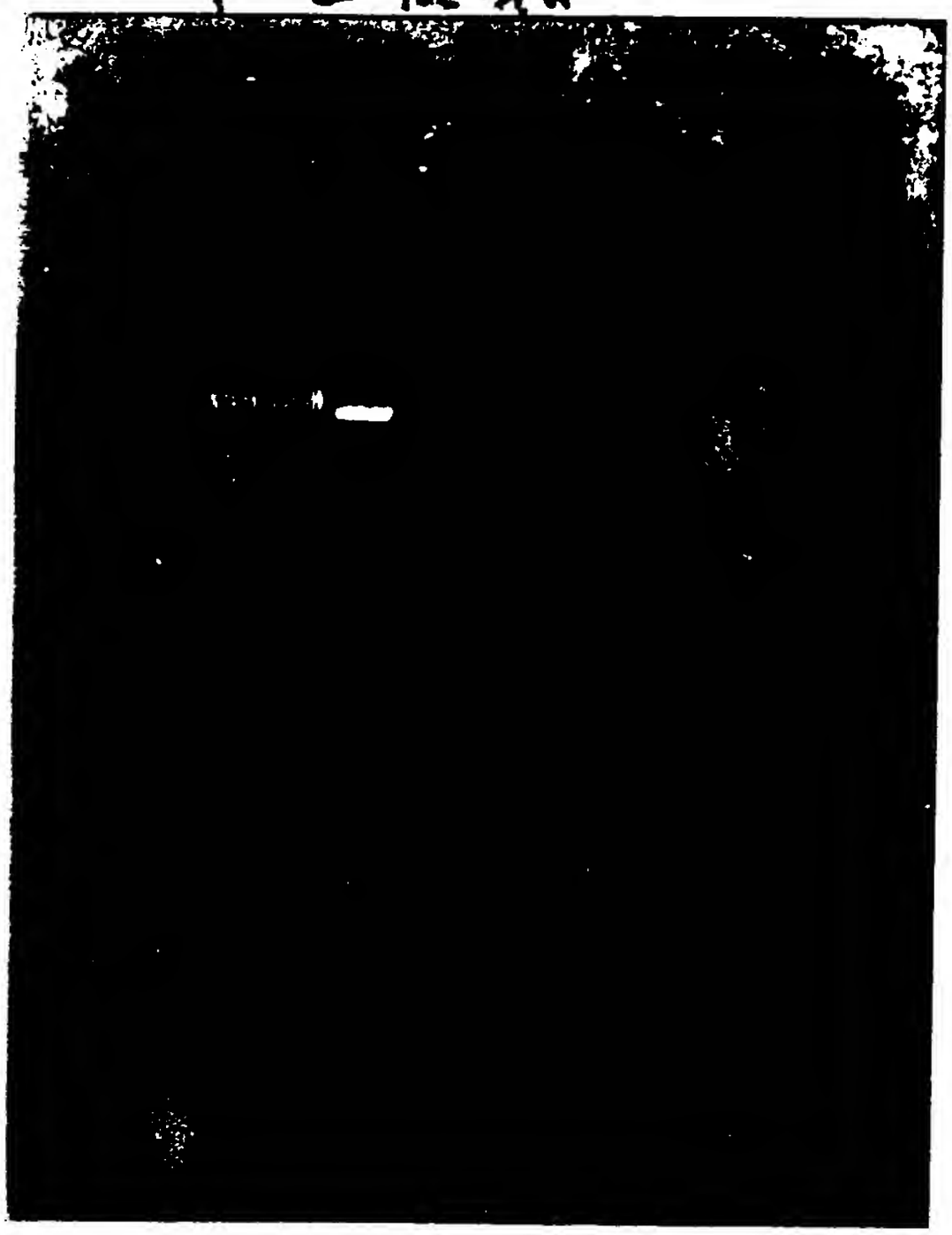
T  dNTP / 3'OH = 1.28 nM / 8.85x10^-5 nM = 14,414

1096 / 526,960 (7.5) (14,414) = 40

G  dNTP / 3'OH = .52 nM / 8.85x10^-5 nM = 5,856

12,940 / 135,996 (7.5) (5,856) = 73

| | # | % |
|---|---|---|
| A | 11 | 7% |
| C | 31 | 20% |
| G | 73 | 47% |
| T | 40 | 26% |
| | | 100% |



A = .07 C = .2 G = .47 T = .26

term = .018382
ala = 9.399999E-02
arg = .111766
asn = .002254
asp = .015134
cys = .056212
gln = 7.560001E-03
glu = .017766
gly = .2209
his = .00644
ile = 9.645999E-03
leu = .088504
lys = .002646
met = 8.553999E-03
phe = .031096
pro = .04
ser = .067134
thr = .014
trp = .057434
tyr = .008372
val = .1222

Goal: - find out min. volume of enzyme
- get DNA for gels
- get DNA for HPLC

1. 1λ PUC13/HIII @ 1μg/1λ — 1μg std
4λ Pool
1.2λ 1:40 dil of Stn mdATP — .06 mM dATP
1λ 1:10 dil of Stn mdCTP — .20 mM dCTP
3.2λ 1:10 dil of Stn mdTTP — .64 mM dTTP
1.3λ 1:10 dil of Stn mdGTP — .26 mM dGTP
1λ α-32P dTTP
3λ 3H dGTP
-TdT
-$H_2O$
25λ total

~~1λ TdT undil~~
~~3λ $H_2O$~~

2 same as 1 but,
1λ α-32P dCTP
3λ 3H dATP

| (A) | (B) | (C) | (D) |
|---|---|---|---|
| 1λ TdT, undil | 5λ 1:10 dil TdT | 3λ 1:10 dil TdT | 2λ 1:10 dil |
| 8.3λ $H_2O$ | 4.3λ $H_2O$ | 6.3λ $H_2O$ | 7.3λ $H_2O$ |

Rxn went for 1½ hours

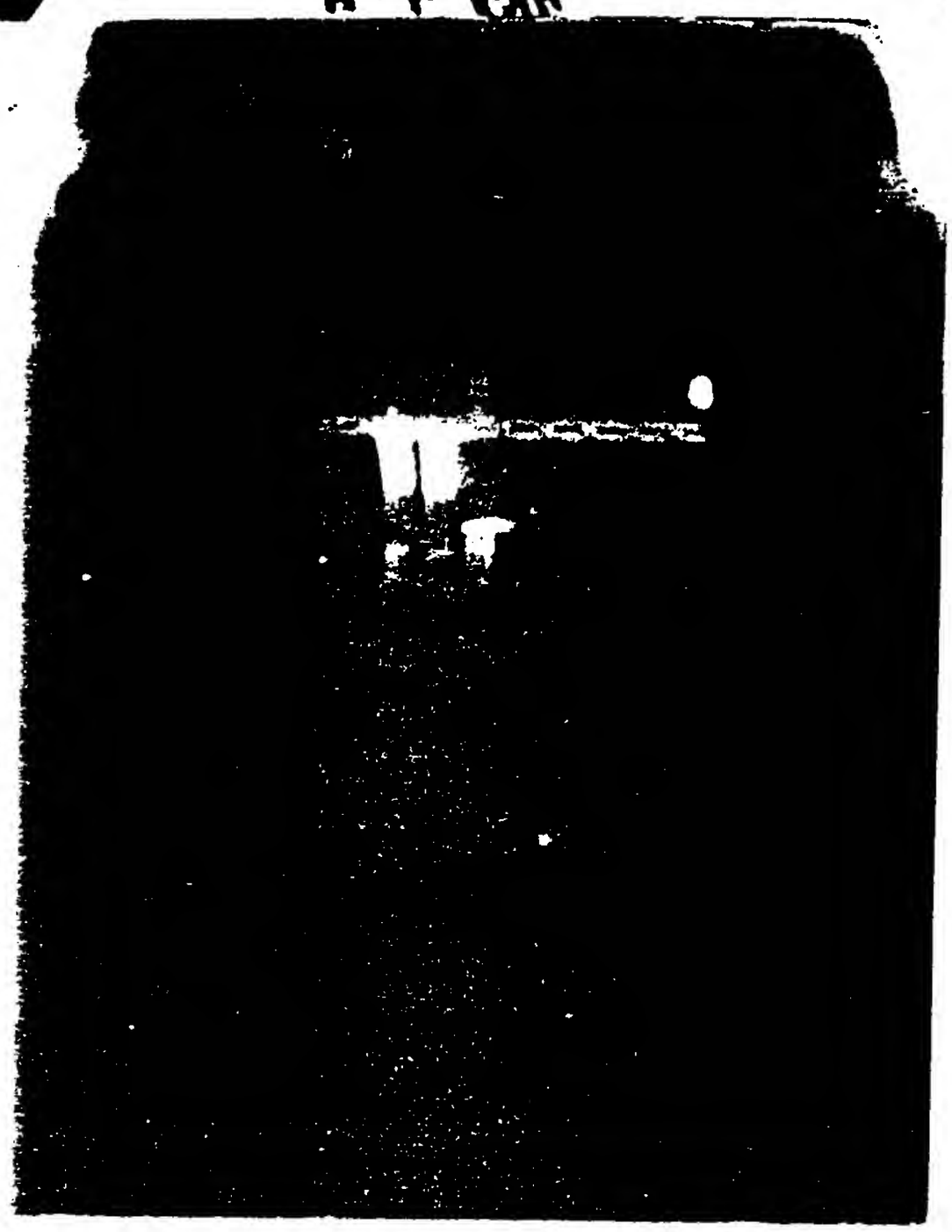
Withdraw 2λ from the rxn mix before adding TdT and spot.

~~After the rxn ends withdraw 13λ and save for HPLC~~

Labelled: A-1 B-1 C-1 D-1
A-2 B-2 C-2 D-2

Aliquot of 12λ was withdrawn from each A-1 & A-2 (6λ on gel, 6λ saved for HPLC) Nothing was withdrawn from B, C, or D

A-1 A-2 Chloride



I gaged: B-2 & A-2 were washed through the filter before they were acidified ⇒ very few ions were absorbed

1: A = H-dATP, B = [illegible]

2: A = $^{3}$H-dATP, B = $^{\alpha-32}$P-dCTP

[illegible] T=000.50 A=[illegible](2.0%) B=[illegible](1.0%) C=153972.4(1.0%) R=3.[illegible]
[illegible] T=000.50 A=[illegible]4(3.0%) B=[illegible](2.0%) C=056230.0(1.5%) R=2.[illegible]
[illegible] T=000.50 A=[illegible].0(2.0%) B=[illegible](1.5%) C=[illegible]3114.3(0.7%) R=2.[illegible]
[illegible] T=000.50 A=[illegible]2(1.5%) B=[illegible].6(0.7%) C=31760.0(0.7%) R=2.[illegible]
[illegible] T=000.50 A=[illegible].8(3.0%) B=[illegible](1.5%) C=020530.0(1.5%) R=2.[illegible]

[illegible] T=000.50 A=[illegible]0(1.0%) B=[illegible]6(1.0%) C=242673.8(0.7%) R=0.[illegible]
[illegible] T=000.50 A=[illegible].0(3.0%) B=[illegible].2(2.0%) C=047170.0(1.5%) R=1.[illegible]
[illegible] T=000.50 A=[illegible].4(1.5%) B=[illegible].0(1.0%) C=271793.6(0.7%) R=[illegible]
[illegible] T=000.50 A=[illegible].6(0.7%) B=[illegible](0.5%) C=[illegible]

[illegible] T=000.50 A=[illegible]0(1.5%) B=[illegible] C=[illegible]
[illegible] T=000.50 A=[illegible].0(0.7%) B=[illegible] C=[illegible]
[illegible] T=000.50 A=[illegible].0(1.5%) B=[illegible] C=[illegible]
[illegible] T=000.50 A=[illegible].0(0.7%) B=[illegible](0.7%) C=[illegible]
[illegible] T=000.50 A=[illegible].0(1.5%) B=[illegible].0(0.7%) C=[illegible]

[illegible] T=000.50 A=[illegible].2(0.7%) B=[illegible].0(0.5%) C=290010.0(0.3%) R=[illegible]

Rxn A

(A) $\frac{(16902)(2)}{(192833)(13)}(1351) = 18$ ✓ unreliable due to gas

(C) $\frac{(38495)(2)}{(646835)(13)}(4505) = 42$ ✓ unreliable due to gas

(G) $\frac{(27340)(2)}{(41680)(13}$ ← $(5856) = 591$

(T) $\frac{(106581)(2)}{(329711)(13)}(14414) = 717$

R n B:

(A) 66,58+
~~35,160~~(2) (1351) = 3 ✓ unreliable-good
~~+80,000~~(23)
248,030)

(C) (192,833)(2) (4,505) = 93 ✓ unreliable-good
(813,210)(23)

(E) (25,160)(2) (5856) = 258
(49,750)(23)

(F) (9,675)(2) (14,414) = 215
(406,142)(23)

R n C

(A) (5,156)(2) (4,505)
(C) 274,033)(23) ~~(13851)~~ = 79

(E) (07,580)(2) (1351) = 59
(14,000)(23)

(G) (5,734)(2) (5856) = 224
(2560)(23)

(I) (10,464)(2) (14414) = 190
26280)(23)

R+A+D

(A) $\frac{72,084\ (2)}{316,971\ (23)}\ (1351) = 27$

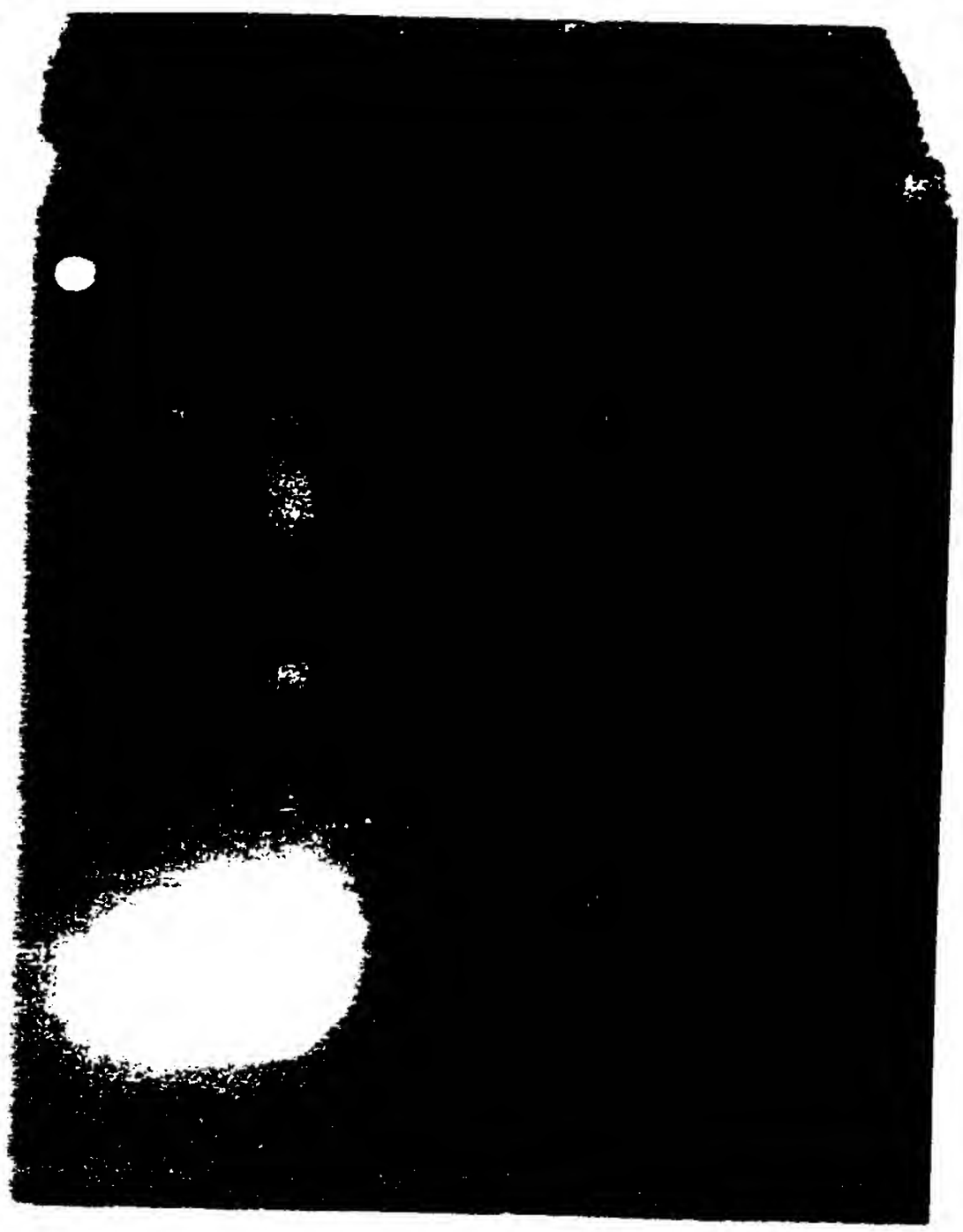
(C) $\frac{(158686)(2)}{(711,080)(23)}\ (4505) = 87$

(G) $\frac{(14,430)(2)}{(41,504)(23)}\ (5856) = 177$

(T) $\frac{(25,521)(2)}{(97,240)(23)}\ (14,414) = 162$

| Results | Replicate Rxn A | | Replicate Rxn B | | Replicate Rxn C | | Replicate Rxn D | |
|---|---|---|---|---|---|---|---|---|
| dNTP | # | % | # | % | # | % | # | % |
| A | 18* | | 3* | | 59 | 10.7 | 27 | 6.0 |
| C | 42* | | 93* | | 79 | 14.3 | 87 | 19.2 |
| G | 591 | sum: 1308 | 258 | sum: 417 | 224 | 40.6 | 177 | 39.0 |
| T | 717 | | 215 | | 190 | 34.4 | 162 | 35.8 |
| | | | | | 552 | 100 | 453 | 100 |

* unreliable due to goof up



Razor → slit orientation slot

DNP? →

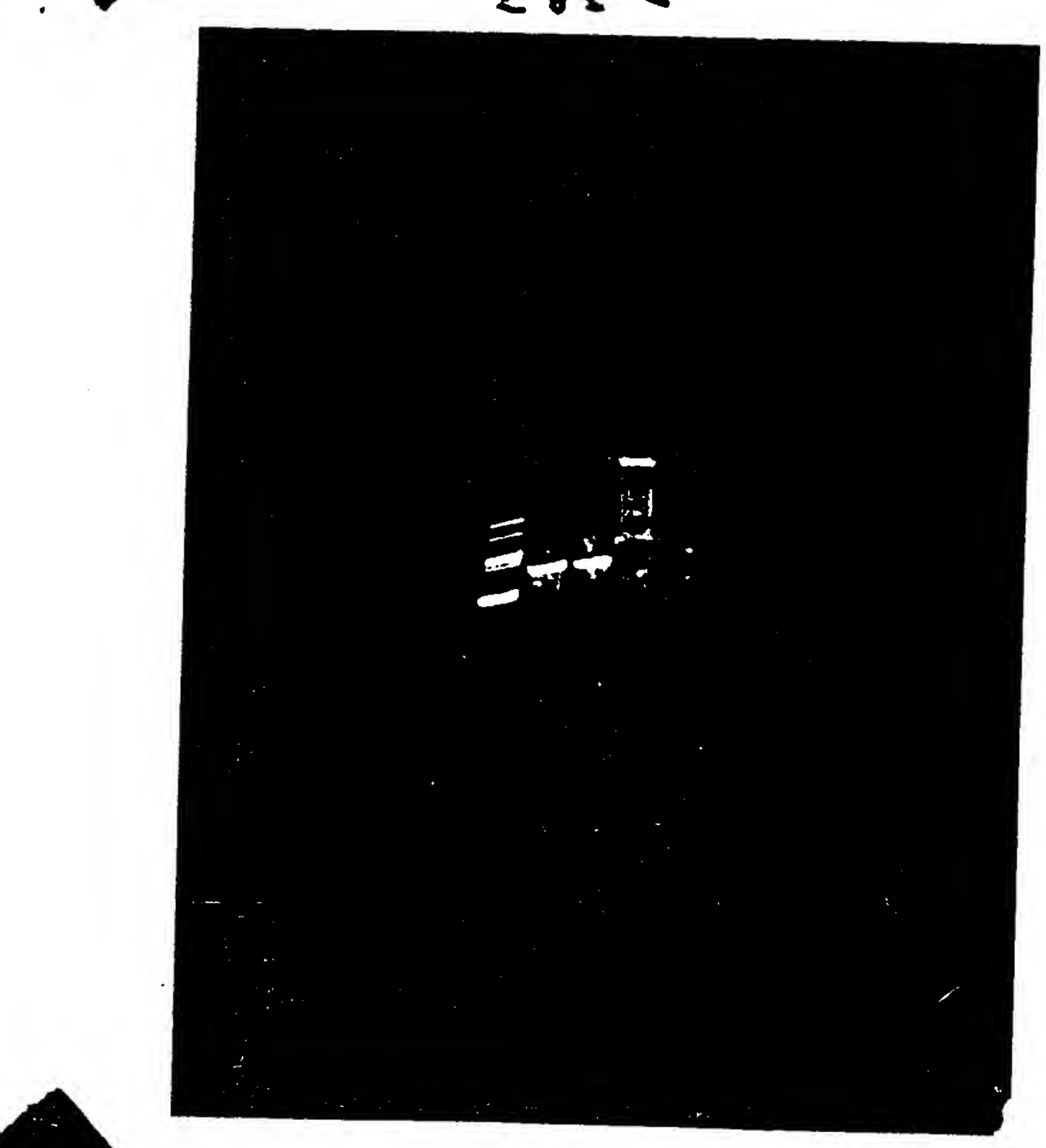
6 & hour autoradiogram of the above gel

PUC13/HIII Digest

190λ PUC13 at 1μg/λ (= 190μg)
~~48~~ 33λ HindIII at 40u/λ (= ~~1920~~ 1320)
26.4λ 10x "Core" buffer 14Z
15λ $H_2O$
264.4λ total

Rxn went O/N @ 37°C

(given 200μg from Ray - 10μg/in 10λ) saved



MS/measure after cut Nuclease A-1

cut w/ Nuclease A-1

After 12 hours the digest was stopped. Together w/ A-Z (from the day before) the DNA was EtOH ppt'd.

The Pvu I/Hind III was reeluted in 190 λ TE

The A-Z was re-eluted & cut w/ EcoRI cut 20 u/λ

17 λ EcoRI at 20 u/λ (= 20 u)
ETOH ppt & dryd A-Z DNA
2 λ 10x High Salt buffer
1 λ $H_2O$
20 λ total

# Checking DNA concentration

| | 13/11 III - batch 1 | 13/11 III - batch 2 |
|---|---|---|
| Nan → 280 | .29 | .138 |
| → (260) | .378 | .277 |

diluted 1:40 (5λ DNA + 195λ TE)

$$\frac{40 \times \text{(260 absorbance)}}{22} = \frac{\text{mg}}{\text{ml}}$$

($\frac{260}{280}$ ratio should be about 2, which it is)

↳ batch-1 .7 µg/λ
batch-2 .5 µg/λ
} ⟹ tail length ~1.5x as long as target

Large scale preparation for TdT step #1

Goal: To make all the reagents up in a sufficient quantity that identical preparations may be used for trial & actual step 1

Large scale set up of assay on p. 24 (note)

1ml of Pool + dNTP at .06nM dATP
.20nM dCTP
.64nM dTTP
.26nM dGTP
} assuming stock concentrations at 50mM (not true) actual val

| Stock | | final concentration in total volum of 1ml |
|---|---|---|
| 100λ | 1M K+ Cacodylate | 100mM Cacodylate |
| 20λ | 50mM $CoCl_2$ | 1mM $CoCl_2$ |
| 40λ | 0.5M ditt / 1:100 dil | 0.2mM ditt |
| 12λ | 50mM dATP / 1:10 dil | .06mM dATP |
| 4λ | 50mM dCTP | .20mM dCTP |
| 12.8λ | 50mM dTTP | .64 mM dTTP |
| 5.2λ | 50mM dGTP | .26mM dGTP |
| 6λ | $H_2O$ | |
| 200λ | total | |

(concentrations are correct when this pool is in a total volume of 1ml ⇒ 5X pool)

must add - Taq
- label
- TdT
- DNA

To make 1ml total of the above pool - use 5 times the quantity

| | | |
|---|---|---|
| ✓ | 500λ | $K^+$ Cacodylate |
| ✓ | 100λ | $CoCl_2$ |
| ✓ | 200λ | 1:100 dil of dNTP |
| ✓ | 60λ | 1:10 dATP |
| ✓ | 20λ | dCTP |
| ✓ | 64λ | dTTP |
| ✓ | 26λ | dGTP |
| ✓ | 30λ | $H_2O$ |

1000λ (And this will be a 5x pool (+) → dNTPs included

Add $CoCl_2$ last (at least after dNTPs)

TdT Assay

1.

✓ 5λ Pool++(5x)

✓ 1.4λ PuCl3(H$^{III}$ - batch 2 at 0.5 µg/λ

✓ 0.5λ TdT

✓ 1λ $^{\alpha-32}$P dTTP

✓ 2λ $^{3}$H dGTP

✓ 15.1λ $H_2O$

25λ total

2. same as #1, but

✓ 1λ $^{\alpha-32}$P dCTP

✓ 2λ $^{3}$H dATP

Take 2λ out to spot before adding TdT

⇒ 2λ (taken out of 24.5)

12:05 pm

8λ withdrawn from both 1 & 2 before being stopped

⇒ 15λ total counted

PUC1 mw = (549)(2686 bp) = 1,743,214 g/mol

$1 \times 10^{-6}$ g $\left(\frac{1}{1,743,214} \frac{\text{mole}}{\text{g}}\right)$

$1\mu g = 5.74 \times 10^{-13}$ Moles

12 3'OH per molecule

$\Rightarrow 1.15 \times 10^{-12}$ 3'OH M

in 1μg

Scroll Down or next page

[A] $\frac{dATP}{3'OH} = \frac{.06mM}{(.7)(4.44\times10^{-5}mM)} = 1,931$

~~$\frac{10,380(2)}{(24,300)(15)}(1931) = 110$~~ $\frac{63,290(2)}{167,258(15)}(1931) =$

[C] $\frac{dCTP}{3'OH} = \frac{.20mM}{(.7)(4.44\times10^{-5}mM)} = 6,435$

$\frac{24,348(2)}{511,642(15)}(6,435) = 405$

[G] $\frac{dGTP}{3'OH} = \frac{.26mM}{(.7)(4.44\times10^{-5})} = 8,366$

$\frac{(10,380)(2)}{(24,300)(15)}(8,366) = 476$

[T] $\frac{dTTP}{3'OH} = \frac{.64mM}{(.7)(4.44\times10^{-5}mM)} = 20,592$

$\frac{39,378(2)}{193,762(15)}(20592) = 558$

| | # | % | (mM) per minute |
|---|---|---|---|
| A | 97 | 6.3% | 5.9% |
| C | 405 | 26.4% | 21.1% |

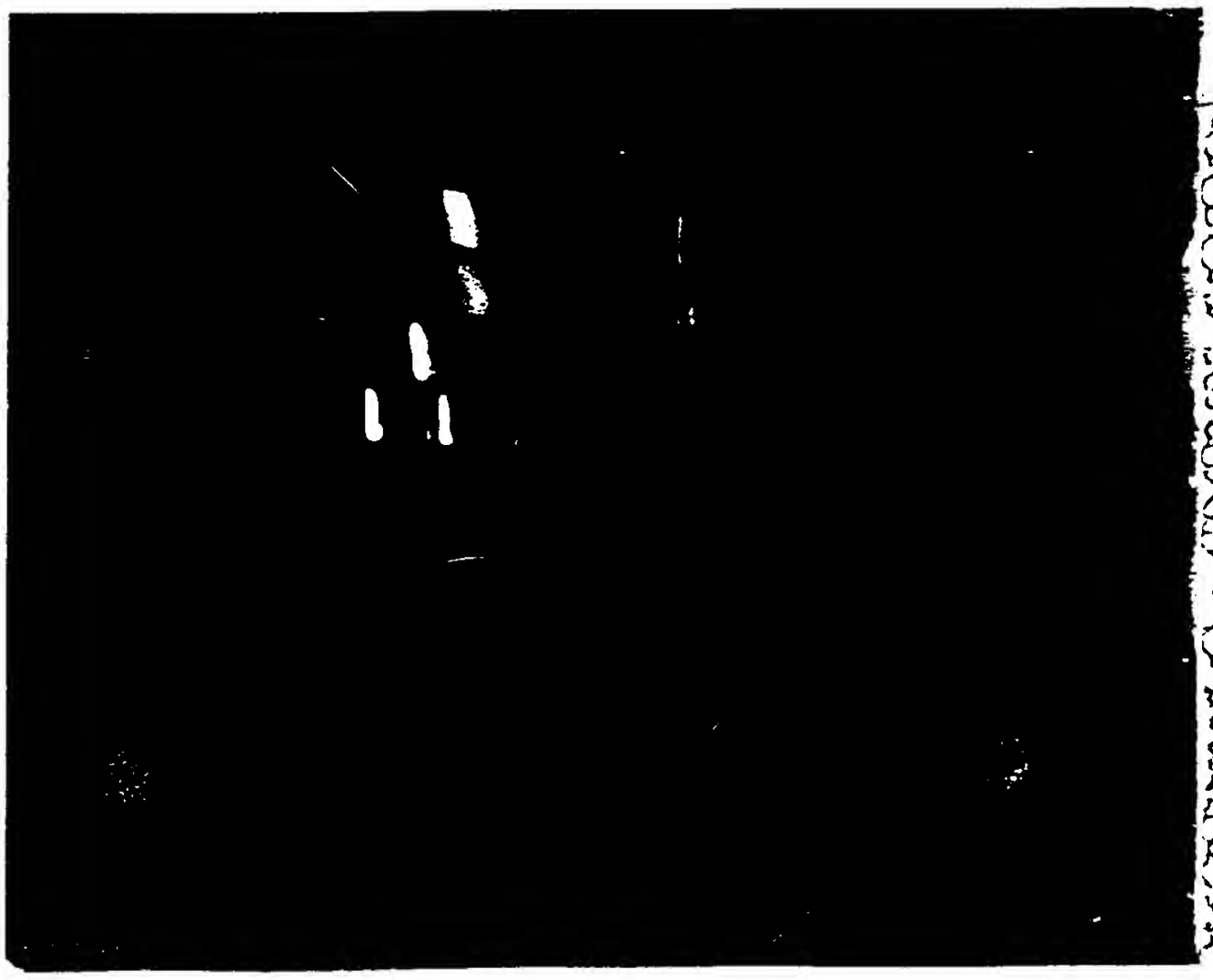
T=000.50 A=010380.0(3.0%) B=039373.0(2.0%) C=052[illegible]

T=000.50 A=063290.0(1.5%) B=241348.0(0.7%) C=3[illegible]

T=000.50 A=024300.0(2.0%) B=193752.0(0.7%) C=25[illegible]

T=000.50 A=167258.0(0.7%) B=511642.0(0.5%) C=870[illegible]

Lamp { – counts/profile
Lamp { – at most 200 counts/profile

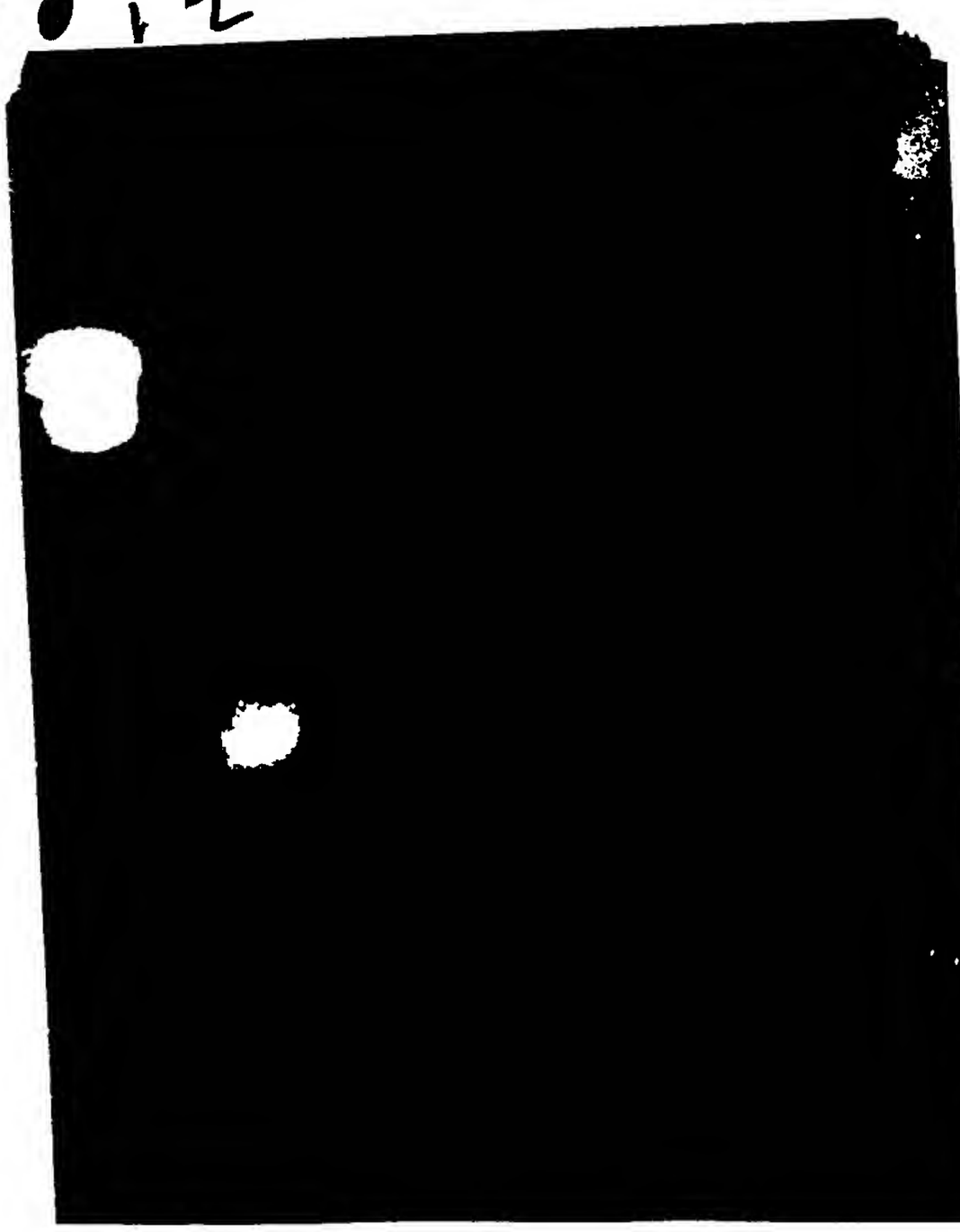


1a
2
13/III
13

A = .063 C = .264 G = .31 T = .363

term = 1.561953E-02
ala = .08184
arg = 8.912469E-02
asn = 2.488563E-03
asp = 1.224531E-02
cys = 7.055631E-02
gln = 6.203736E-03
glu = 7.28469E-03
gly = .0961
his = 1.042826E-02
ile = 1.577961E-02
leu = .1449818
lys = 1.480437E-03
met = 7.08939E-03
phe = 8.261916E-02
pro = 6.969601E-02
ser = .1080773
thr = .016632
trp = .0348843
tyr = 1.433886E-02
val = .11253

012



razor index

44 hour autoradiograms

TdT assay w/less enzyme - pilot for large-scale prep

| | | |
|---|---|---|
| 5λ | pool tt (5x) | st'd |
| 1.4λ | PUC 13/HIII - batch 2 at 0.5μg/λ | .7μg |
| 1λ | α-32 PdTTP | |
| 2λ | $^3$H dGTP | |
| 1λ | 1:3 dil of TdT | |
| 14.6λ | $H_2O$ | |
| 25λ | total | |

2. Same as above, but
   1λ α-32 PdCTP
   2λ $^3$H dATP

- Take 2λ out to spot before adding TdT
  ⇒ (2λ taken out of total volume of 24λ)

- Withdrew 8λ from each (#2 before stopping rxn ⇒ 15λ counted

rxn went for ½ hour

001 T=000.50 A=062396.0(1.5%) B=025949.0(2.0%) C=068422.0(

002 T=000.50 A=026084.0(2.0%) B=052510.0(1.6%) C=090748.0(

003 T=000.50 A=239512.0(0.7%) B=228418.0(0.7%) C=684198.0(

004 T=000.50 A=175398.0(0.7%) B=273325.0(0.5%) C=684198.0(

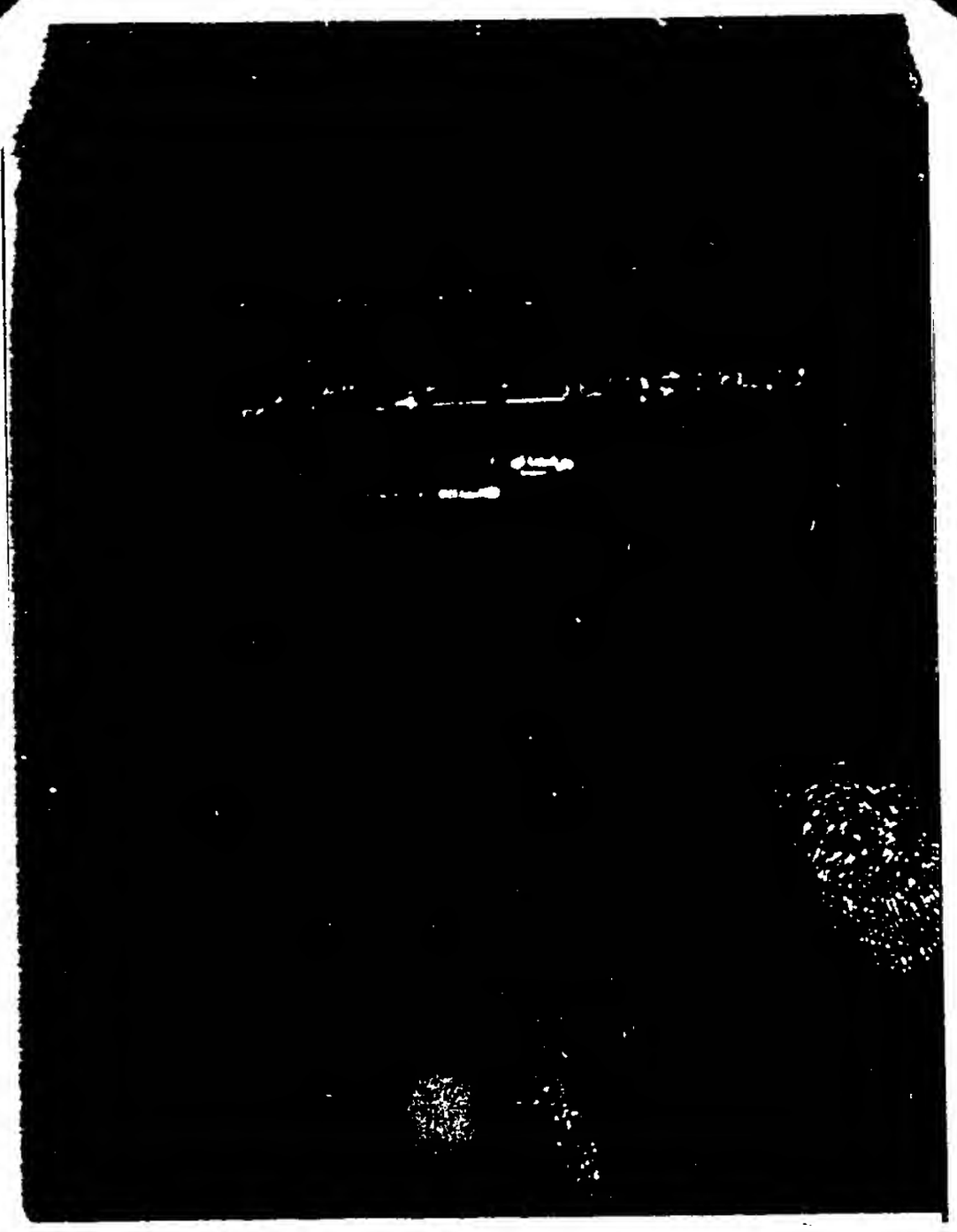
[A] $\frac{26,084\ (2)}{176,398\ (15)}\ (1931) = 38$

[C] $\frac{52,510\ (2)}{373,324\ (15)}\ (6,435) = 121$

[T] $\frac{62,396\ (2)}{239,512\ (15)}\ (8,336) = 290$

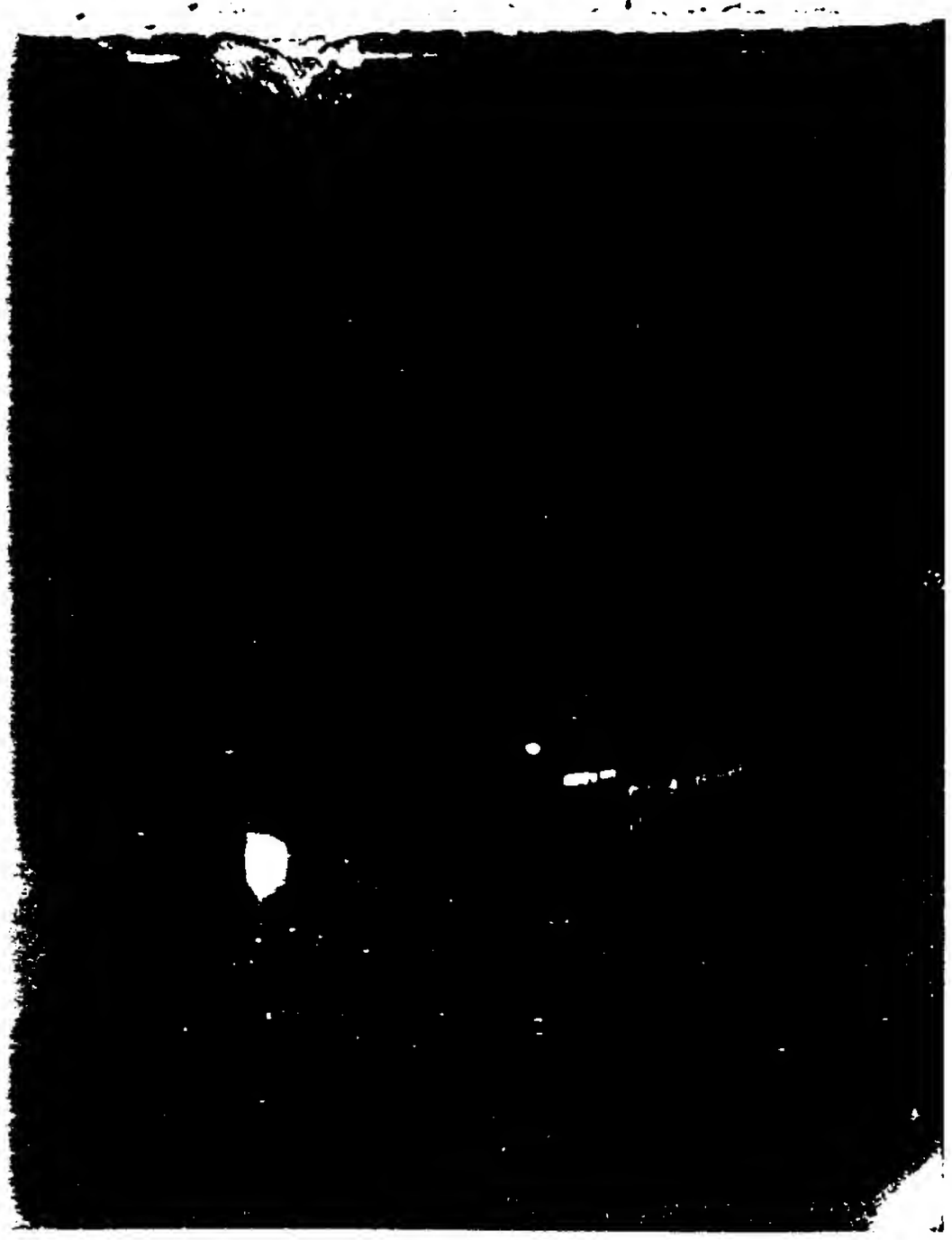
[T] $\frac{26,948\ (2)}{268,618\ (15)}\ (20,592) = 275$

| | # | % |
|---|---|---|
| A | 38 | 5.2% |
| C | 121 | 16.7% |
| T | 290 | 40.0% |
| T | 275 | 38.0% |



~~The sample should be ~3x as concentrated, but it does not look it.~~

~3x as much cut added as sent



175/1.4 = 125 x scale-u

# Large Scale Random TdT Tailing

175λ [redacted] PuC13/H$^{III}$ at 0.5 μg/λ  87.5 μg DNA
625λ 5X pool$^{++}$  std
50λ TdT ($\frac{1}{2.5}$ of the 1λ in 25λ)
[redacted]

(12 [redacted] 25λ PuC13/H$^{III}$ at 0.5 μg/λ  89.0

1. ✓ 87.5λ PuC13/H$^{III}$ at 0.5 μg/λ  43.75
✓ 312.5λ 5X Pool$^{++}$  std
✓ 25λ TdT ($\frac{1}{2.5}$ of the 1λ in 25λ)
✓ 10λ $\alpha$-32 PdTTP
✓ 20λ $^3$H dGTP
✓ 1107.5λ $H_2O$
1562.5λ total

2. Same as above, Incl
10λ $\alpha$-32 PdCTP
20λ $^3$H dATP

---

- Before Adding TdT withdraw [redacted] 10λ from each tube and spot
- After rxn ends (1 hour) withdraw 20λ from each tube - place [redacted] in culture tube - for counting
  - withdraw 7λ and place in eppendorf - this is for gel
- The remaining bulk should be chilled / phenol extracted / EtOH ppt.

Did I use the true concentration
of the dNTPs in this calculation
& did I just take them to be 50mM

P2014 T=000.50 A=140868.0(1.05) B=36858.0(0.52) C=837610.0(6.24)

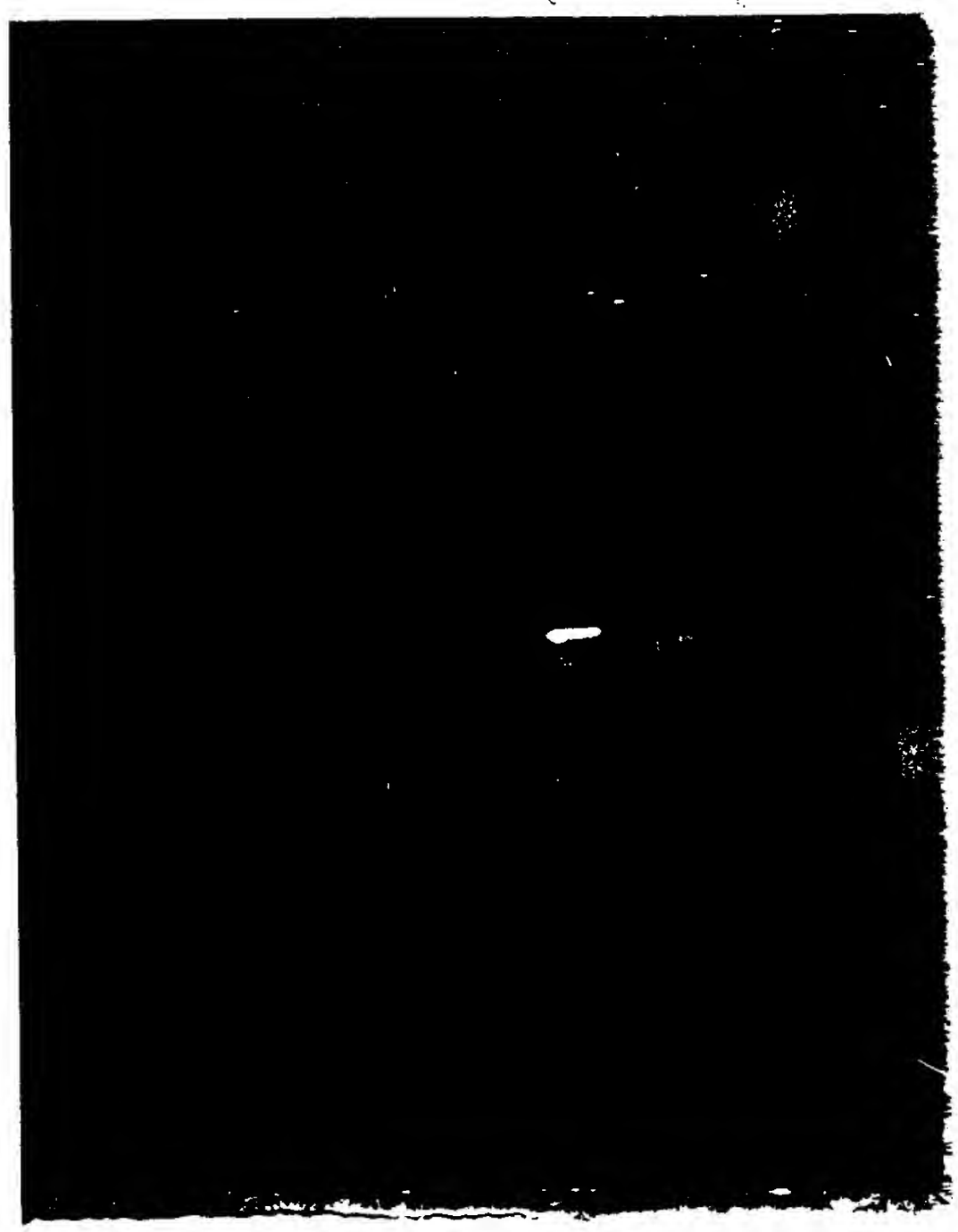
A $\frac{11,106}{140868(2)}$ (1931) = 76

C $\frac{32,122}{36858(2)}$ (6,435) = 286

G $\frac{15,440}{153372(2)}$ (8,336) = 420

I $\frac{5,142}{141,982(2)}$ (20,592) = 373

| | # | % |
|---|---|---|
| A | 76 | 6.6% |
| C | 286 | 24.8% |
| G | 420 | 36.4% |
| T | 373 | 32.3% |
| total | 1155 | 100.1% |



A = .065 C = .248 G = .364 T = .323

term = 1.664903E-02
ala = 9.027199E-02
arg = .1004221
asn = 2.412475E-03
asp = 1.350986E-02
cys = 6.713361E-02
gln = 6.91548E-03
glu = 1.015014E-02
gly = .132496
his = 9.20452E-03
ile = 1.335282E-02
leu = .1248611
lys = 1.812525E-03
met = 7.64218E-03
phe = 5.957186E-02
pro = .061504
ser = 9.361386E-02
thr = .01612
trp = 4.279621E-02
tyr = 1.198815E-02
val = .117572

#1 eluted in ~ 110%
#2 eluted in ~ 80%

| λ(nm) | Sample 1 | 2 |
|---|---|---|
| 260 | .104 | .269 |
| 280 | .054 | .160 |

← 1/100 dilution (2λ in 198λTE

320

$$\frac{40X}{22} = \frac{mg}{mL} \quad (A_{260})$$

1 ⇒ $\frac{100}{40(.104)}{22}(100)$ = 18.9 $\frac{mg}{mL} = \frac{\mu g}{\mu L}$ (dil. factor)

2 ⇒ $\frac{100}{40(.269)}{22}(100) =$ 49 $\frac{mg}{mL} = \frac{\mu g}{\mu L}$

Since these solutions are visibly dark

⇒ re-prep/dilute

dilute to total volume of 200λ for both 1 & 2

After 2nd phenol extraction/EtOH ppt:

| | 1 | 2 |
|---|---|---|
| 260 | .60 | .49 |
| 280 | .28 | .23 |
| 320 | ϕ | .04 |

1:200 dil

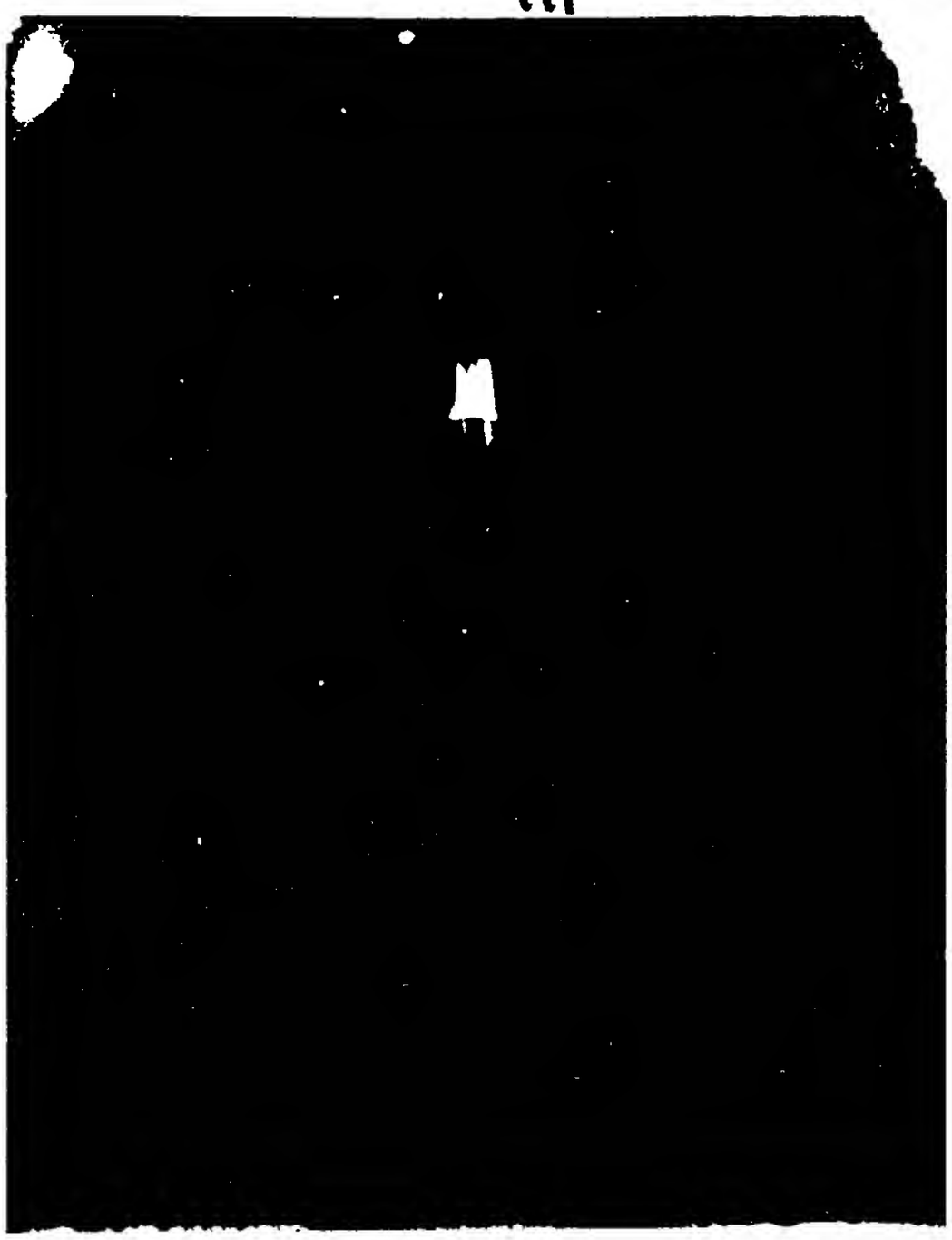
X = 260 reading

[1] ⇒ $\frac{200(.60)}{22} = 5.5 \frac{mg}{ml} = \frac{\mu g}{\lambda}$ (165 μg total in 30λ)

[2] ⇒ $\frac{200(.49)}{22} = 4.5 \frac{mg}{ml} = \frac{\mu g}{\lambda}$ (135 μg total in 30λ)

above in a total volume of ~30λ per 1 st pen R

1 2 13/14 EE



Following 2 phenol/EtOH treatments (should be same as on p. 37 write, but obviously isn't!)

Pvc19 check of concentration

260 .353
280 .183
320 ~~0~~

dilution 1:100

$\frac{100(.353)}{22} = 1.6\ \mu g/\lambda$ (~65λ total)

Digestion of 19 w/ HindIII

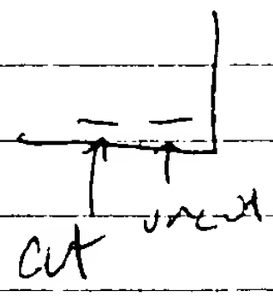
Set aside 5λ uncut in a separate vial in original DNA binary vial (96μg)

60λ DNA @ 1.6 μg/λ (= 96 μg)
50λ HindIII @ 10U/λ (= 500 units : 2.5μg of BRL reddi BRL
12.2λ 10X core buffer
122λ

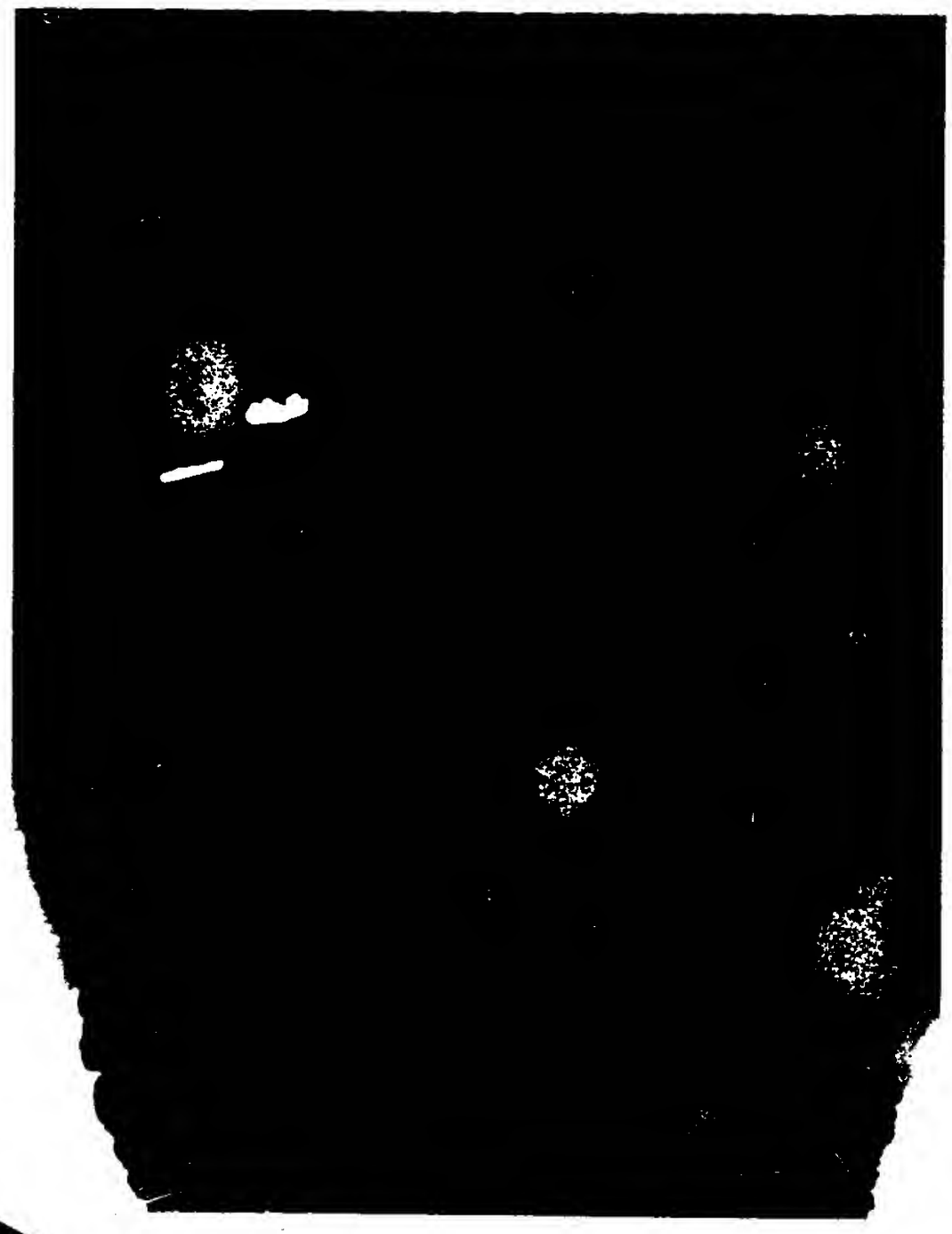
incubated at 37°C

@ 10.10am

After 4½ hours an aliquot (0.5λ) was run out on a gel



Puc 19/14III



after 4 hrs ⇒

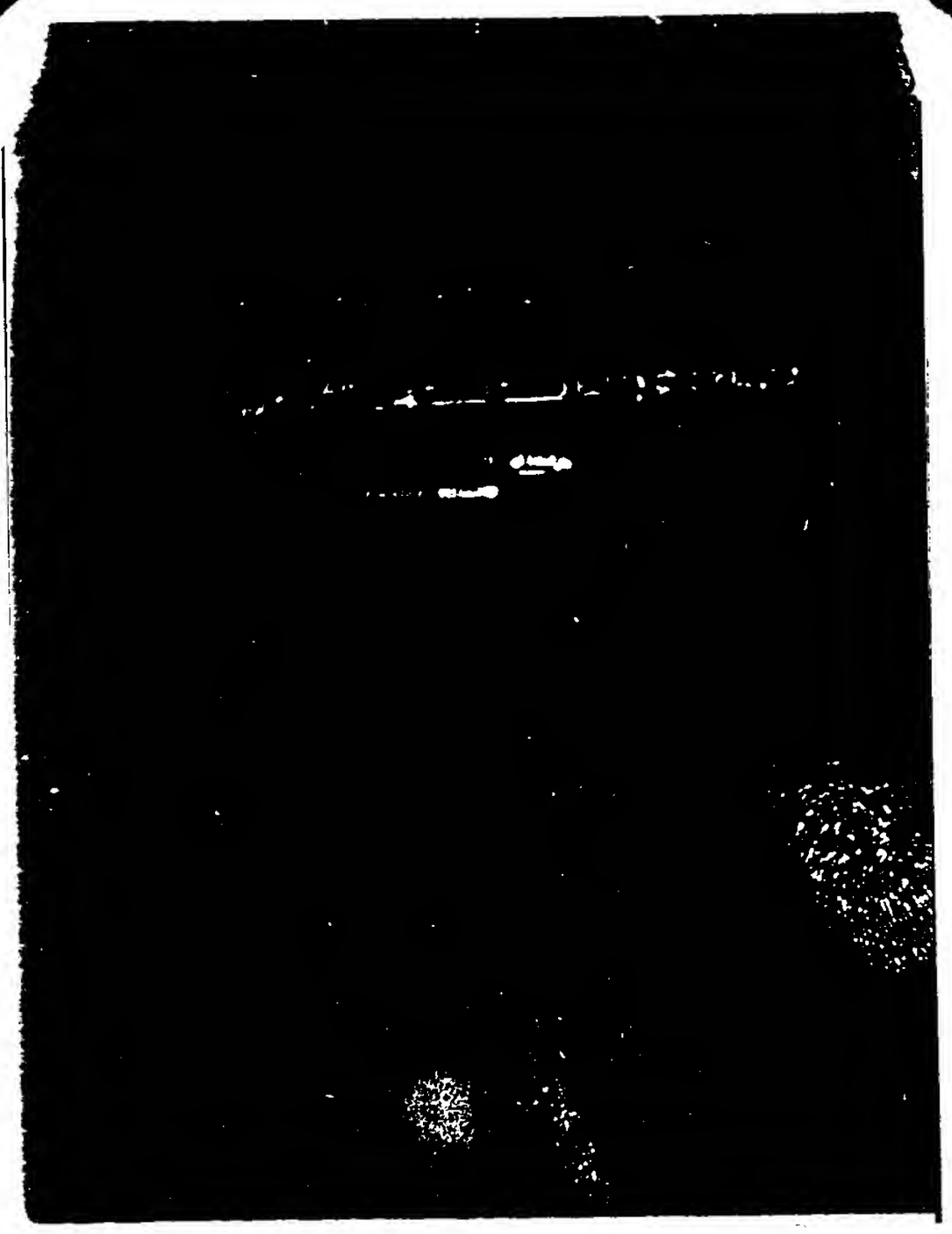
FXN allowed to continue for 10 hours then phenol/EtOH'd and reeluted in total of 600 TE (some spilled)

13-uncut
19-uncut
19/III-

| | Puc19/14III |
|---|---|
| 260 | .118 |
| 280 | .053 |
| 320 | 0 |

1:200 dil

$\frac{100\,(.118)}{} = .54\,\mu g/\lambda$



The ~~uncat~~ if should be ~~~3x as~~
~~Concentrated, but it does not look like it.~~
~3x as much cat added as meat

## TdT Random Tailing Pilot Rxn w/ PUC19

(similar to p.34 yellow)

| | | |
|---|---|---|
| ✓ 1. | 5λ pool ++ (5x) | St'd |
| ✓ | 1.3λ PUC19/HIII at .54μg/λ | .7μg |
| | 1λ $^{\alpha-32}$P dTTP | |
| | 2λ $^{3}$H dGTP | |
| | 1λ 1:2.5 dil of TdT | |
| ✓ | 14.7λ $H_2O$ | |
| | 25λ total | |

2. Same as above, but

1λ $^{\alpha-32}$P dCTP
2λ $^{3}$H dATP

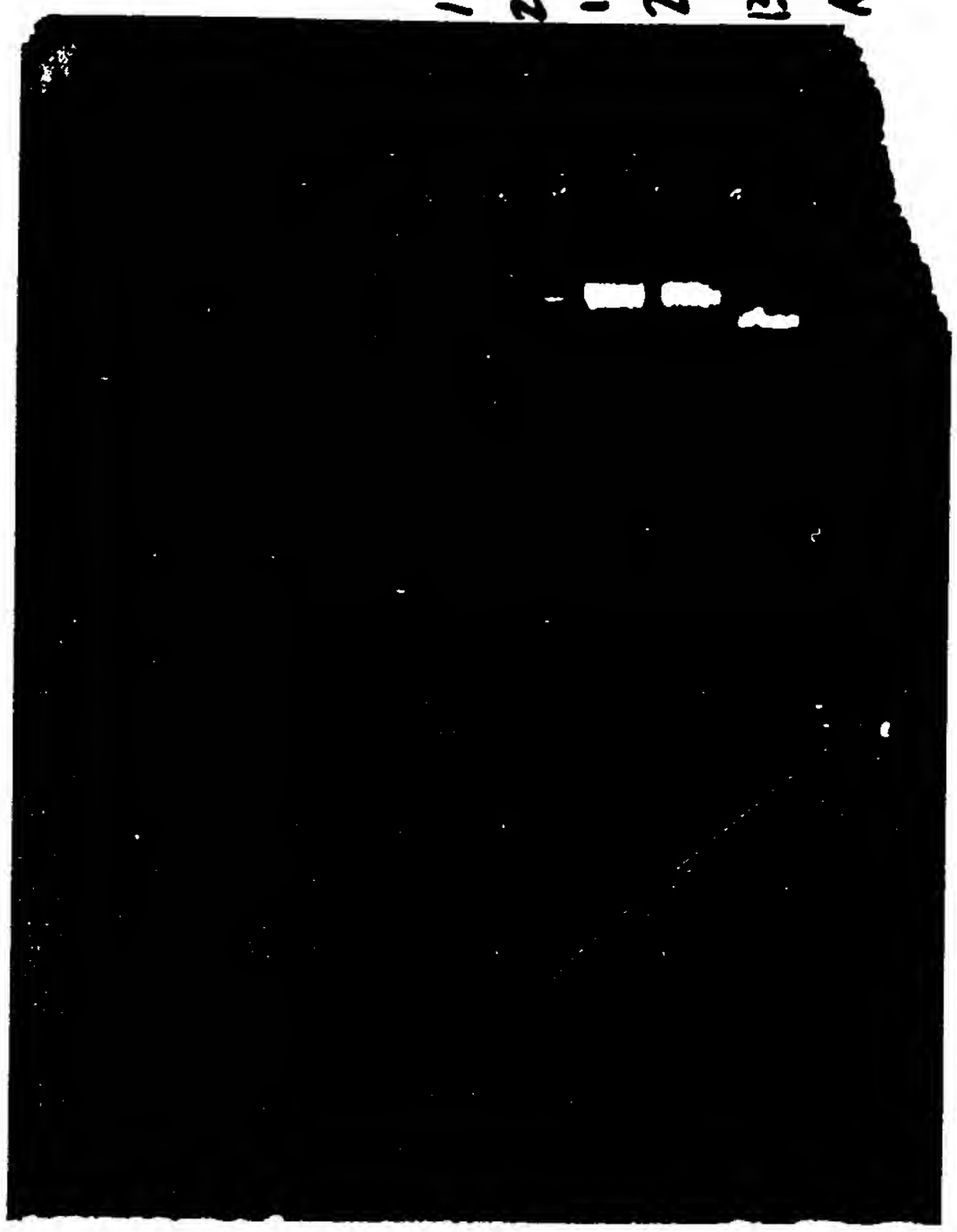
- Before Adding TdT Spot 1λ of the rxn mixture
- After Rxn is over withdraw 5λ and place in Separate culture tubes - these will be counted - the remaining 19λ should be chilled (& stored for purification methods)

10:45 am → 11:30 am
⇒ 45 minutes

Unfortunately, I forgot to change the filter between 1 & 2, so both counts were combined onto a single filter. About the only thing I can conclude is that the rxn worked

101° T=000.62 A=056764.5(1.5%) B=029917.7(1.0%) C=191385.4(1.0%) R=1.760
210 T=000.20 A=052200.0(2.0%) B=065355.0(2.0%) C=143760.0(1.5%) R=1.251
220 T=000.20 A=062800.0(2.0%) B=153300.0(1.5%) C=230320.0(1.0%) R=2.51

Of the 19λ remaining, 3λ of each was saved (for an unpurified gel) and the other 16λ was phenol extracted/EtOH ppt'ed and re-eluted in 12λ each (per tube)
DE




N ZX w/ much greater'd DNA sample as non-extracted sample added (Even less Concentrated relative to non-extracted from gel would suggest)

# TdT purification scheme Trial

Goal: To make TdT to test various methods of purification following IEM

1. ~~[illegible]~~ pool ++ (5X) — s't'd
   ~7λ PuC13/HIII · at 0.5μg/λ — 3.5μg
   1.5λ TdT
   1λ α-32PdTTP
   ~~6.5λ~~ 15λ $H_2O$
   10λ TOTAL
   ↑ incubate at 37°C for 1½ hrs

This should have been 125λ total ⇒ bases more concentrated (should not make too much) a diff since this exp. is aimed at separating labeled pumicl from dNTPs and Enzyme is not to be measured or seen on visualization on a gel

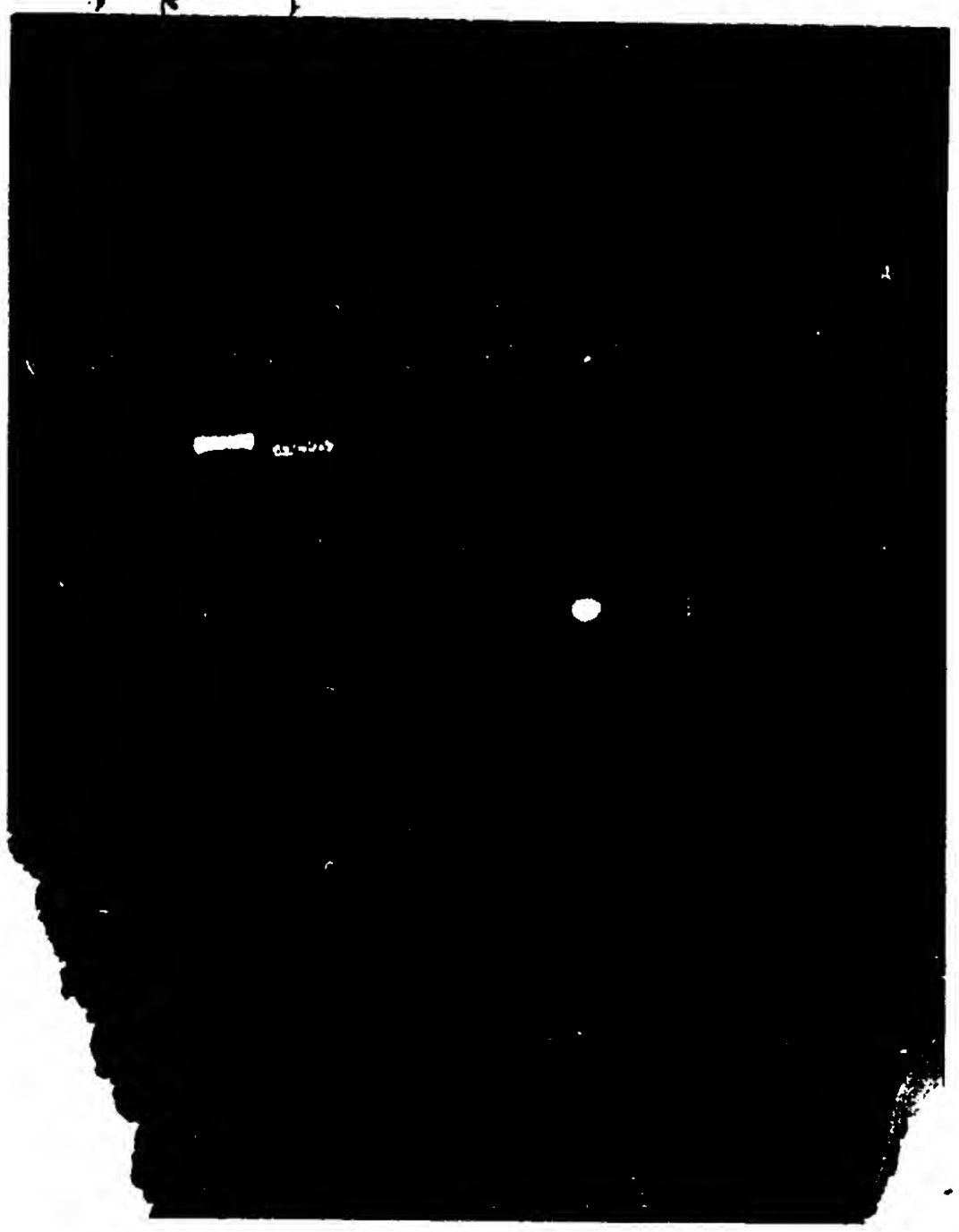
25λ withdrawn & worked up as regular BRL prep

↓

After Etoh ppt
resuspended in 10λ TE — 6λ of this run on gel
w/

- 6λ Nucs purified 13/III trial (25λ → Nucs → 10λ) (6 this)
- 6λ running
- 1λ PuC13/HIII

Nacs
Kawamura
White



Mucking the "Nacs" floated away upon loading because there was still ethanol not yet dryed from the pellets. (And there was a low ratio of loading buffer)

The "Nacs" was completely cold upon loading ⇒ all the dNTP's were gone

(By contrast the phenol/EtOH samples are still pretty hot ⇒ dNTP's present)

Preparation of NACS

1 gram of NaCS 52
10 ml 2M NaCl TE

- mix gently for 1 hour
- allow matrix to settle for 1 hour - remove SN by cup
- repeat until SN is clear (~ one more time)
- store at 4°C until

25 ng of the previous days sample was loaded in a 1cc syringe NAX column, eluted in 4 SN, ~~200~~ ppt, redissolved in 25 ul. 6 ul (of 5 loading buffer) of the NAX was combined w/ an equal amt (& equiv. concentration) on a gel

"nax"
"raw"

→ get AC DNA recovered, at least some appeared on gel (no bottom for the picture)

# Large Scale Random Tail w/ Puc 19

42.3% scaled

55λ Puc19/H$^{III}$ at .54μg/λ 29.7μg
212λ 5X pool$^{++}$ std
16.9λ TdT
~~1058λ~~ clear
$H_2O$
1058λ Total

1. 27.5λ Puc19/H$^{III}$ at .54μg/λ 14.9μg
106λ 5X pool$^{++}$ std
8.5λ TdT
5λ $^{\alpha-32}$P dTTP
10λ $^{3}$H dGTP
372λ $H_2O$
~~529λ~~ total
529λ

2. Same as above but
5λ $^{\alpha-32}$P dCTP
10λ $^{3}$H dATP

1hour at 37°C

---

- Before Adding TdT withdraw 5λ from each tube & spot as the pool
- After rxn ends (1 hour) Withdraw 10λ from each tube to save for counting
- The rest is gel loaded for purif.

13/III 12

193 T=000.50 A=017552.0(3.0%) B=004162.0(5.0%) C=020592.0

194 T=000.50 A=007704.0(5.0%) B=029448.0(6.0%) C=043638.0

195 T=000.50 A=120550.0(1.0%) B=087418.0(1.0%) C=257168.0

196 T=000.50 A=063906.0(1.5%) B=290984.0(0.7%) C=456868.0

[A] $\frac{.7704}{63906(2)}(1931) = 116$

[C] $\frac{29,448}{290,984(2)}(6,435) = 326$

[G] $\frac{17,552}{120,550(2)}(8,336) = 607$

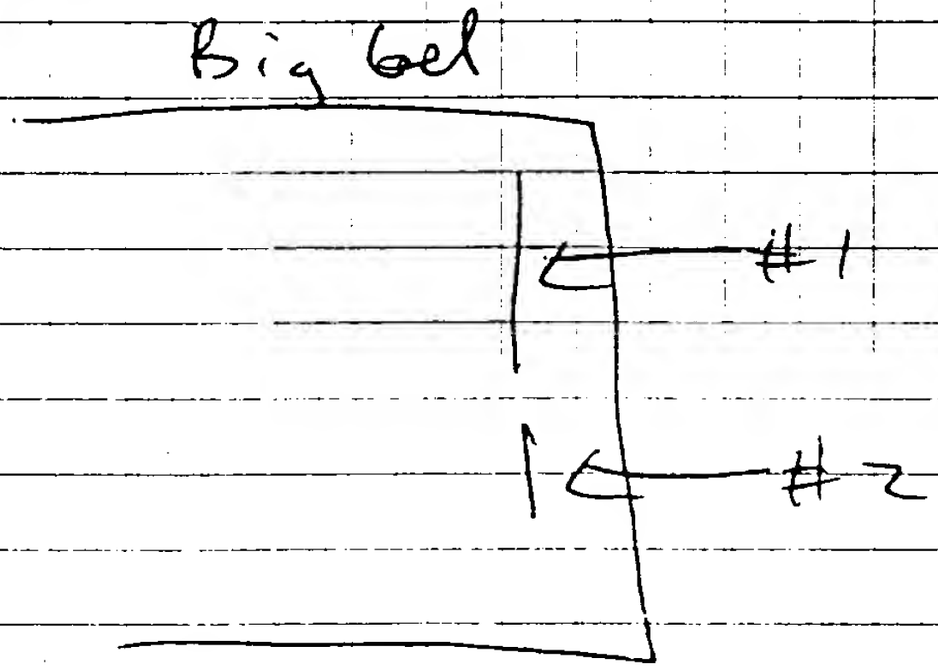
[T] $\frac{4162}{87418(2)}(20592) = 490$

| | # | % |
|---|---|---|
| A | 116 | 7.5% |
| C | 326 | 21.1% |
| G | 607 | 39.4% |
| T | 490 | 31.8% |
| | 1539 | 99.8 |

A = .075 C = .212 G = .395 T = .318

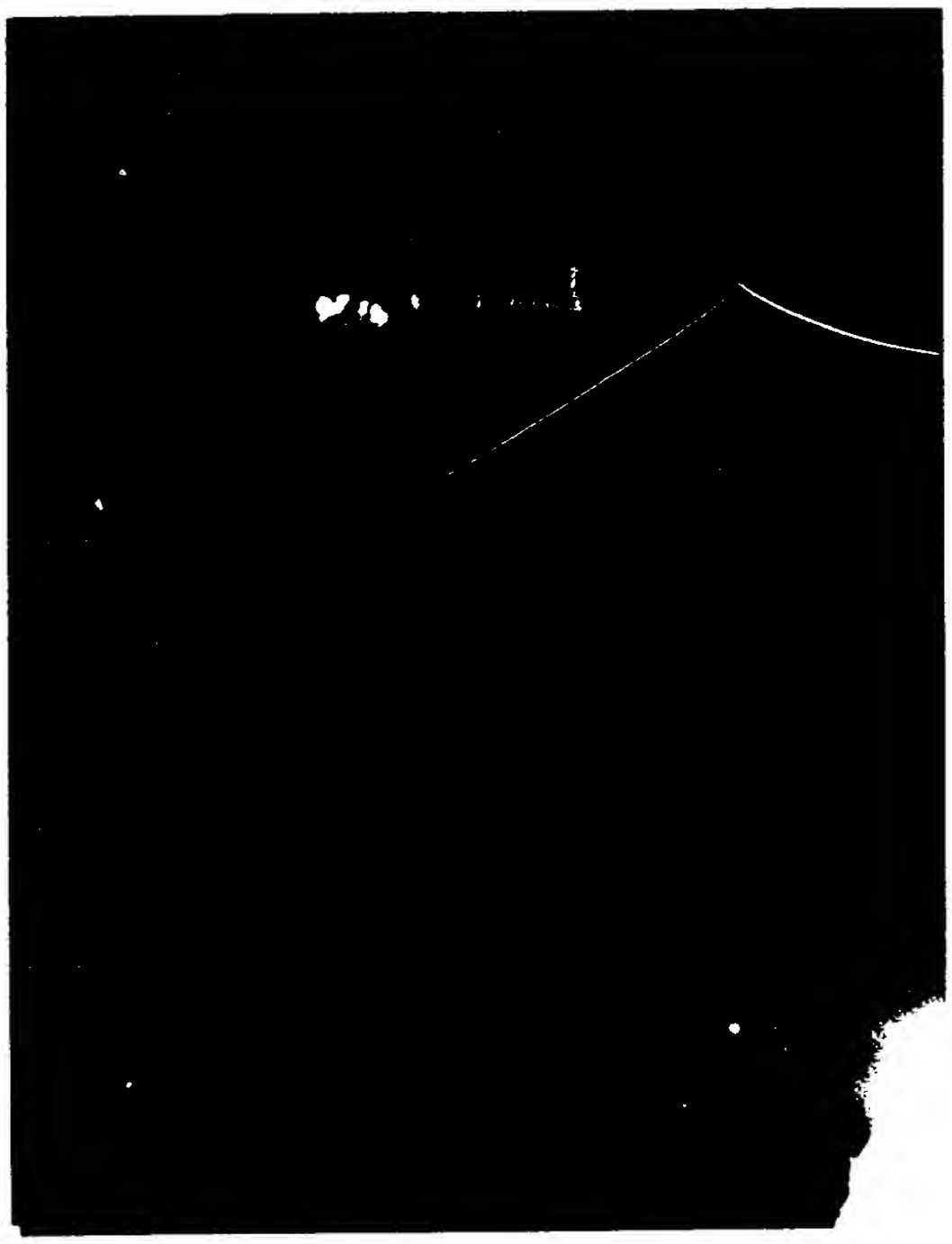
term = 2.063025E-02
ala = 8.374001E-02
arg = 9.766374E-02
asn = 2.98125E-03
asp = 1.570125E-02
cys = .0665733
gln = 7.473001E-03
glu = 1.392375E-02
gly = .156025
his = 8.426999E-03
ile = 1.442925E-02
leu = .1149443
lys = 2.64375E-03
met = 9.42075E-03
phe = 5.359572E-02
pro = .044944
ser = 8.311725E-02
thr = .0159
trp = 4.961595E-02
tyr = .0126405
val = .12561

Big Gel



Purified according to Ray's Protocol, #1 & #2
each eluted in ~1130λ TE

Pur 13/MM
1 - M-tails
2



| Nm | 1 | 2 |
|---|---|---|
| 260 | .165 | .150 |
| 280 | .122 | .100 |
| 320 | .036 | .017 |

1:40 dilution

1 ⇒ $\frac{40(.165)}{22} = .3 \mu g/\lambda$

[redacted]

another way to do calc.
~~can do~~ people

1,552(52.9) = 928,500
total counts putin

1833(80) = 146,640

293,250 ⇒ 1/3 of counts recorded
928,500

146,640 ⇒ ~16%
928,500

2λ from both 1 & 2 axis dotted on filter, dryed & counted

$^3H$ $^{32}P$

T=001.00 A=001833.0(5.0%) B=000399.0(1.5%) C=002200.0(5.0%) D=0

T=001.00 A=001523.0(7.0%) B=000520.0(3.0%) C=001454.0(3.0%) S=1

2λ counted

From counts taken on sample following tailing RXN

1' $^3H$ ⇒ $\frac{17,552 \text{ cpm}}{10\lambda} = \frac{1,755 \text{ cpm}}{\lambda}$.

10λ counted

The above ⇒ $\frac{1,833 \text{ cpm}}{2\lambda} = \frac{917 \text{ cpm}}{\lambda}$

1,785

$\frac{917}{1,755} = .52$ x as concentrated

$^{32}P$ ⇒ $\frac{4162 \text{ cpm}}{10\lambda} = \frac{416 \text{ cpm}}{\lambda}$

$\frac{400 \text{ cpm}}{2\lambda} = \frac{200 \text{ cpm}}{\lambda}$

$\frac{200}{416} \cong .5$X as concentrated as original sample

original sample had $\frac{14.9\,\mu g}{527} = .028\,\frac{\mu g}{\lambda}$

following gel extraction $= \frac{.025\,\mu g}{2\lambda} = .014\,\frac{\mu g}{\lambda}$

(16 $\lambda$ contains 2.24 $\mu g$ DNA)

---

(2) $^3H$: $\frac{7,704\ cpm}{10\lambda} = 770\,\frac{cpm}{\lambda}$

$\frac{1543\ cpm}{2\lambda} = 771\,\frac{cpm}{\lambda}$

$\frac{771}{770} \simeq$ the same concentration as original sample

$^{32}P$: $\frac{29,448\ cpm}{10\lambda} = 2,944\,\frac{cpm}{\lambda}$

$\frac{4529\ cpm}{2\lambda} = 2,265\,\frac{cpm}{\lambda}$

(4) about the same concentration as original PM mix

orig concentration:

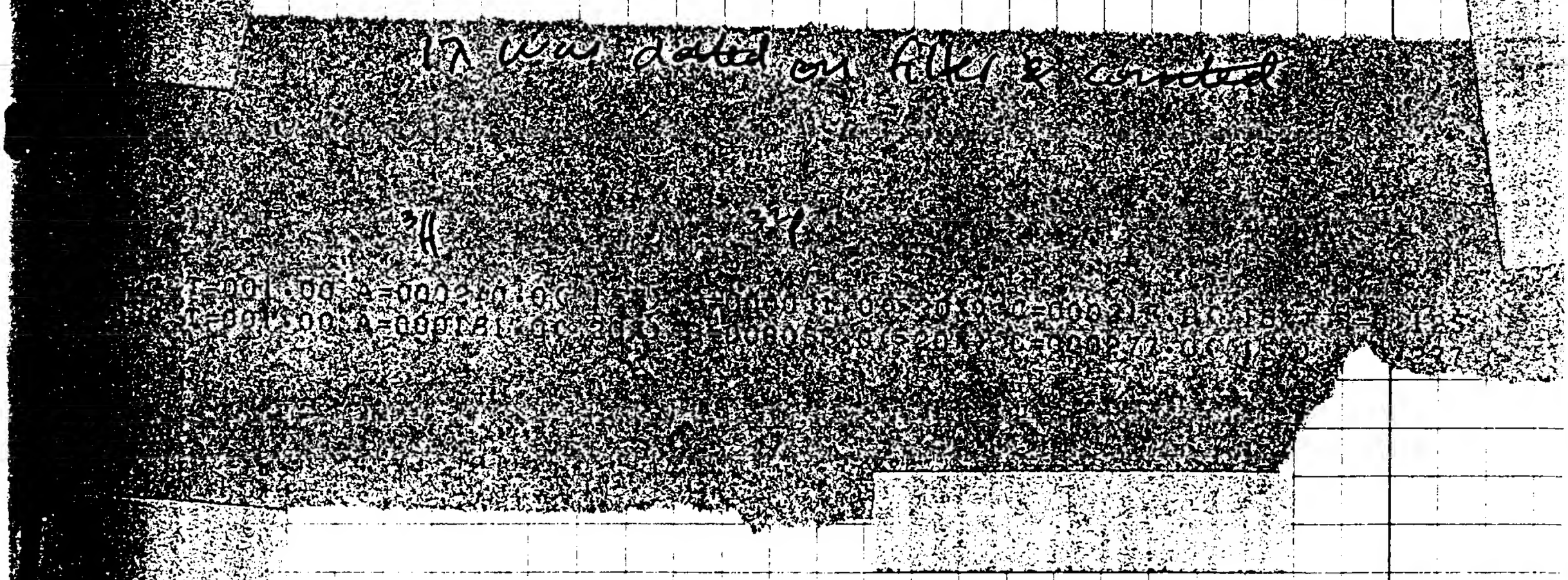
$\frac{14.9\mu g}{529\lambda} = \frac{.028\mu g}{\lambda}$

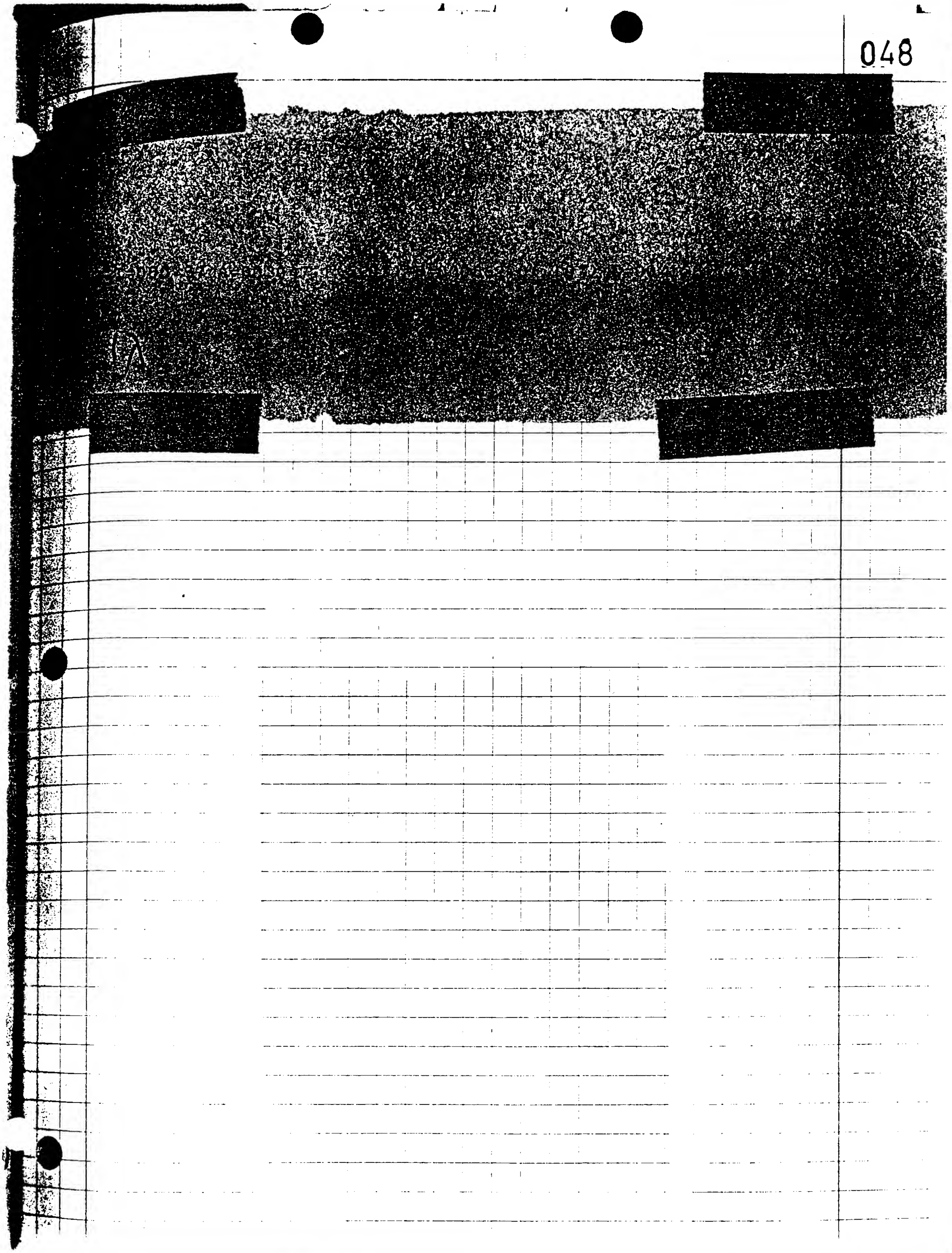
So, purified DNA ~ .028μg
(160λ ⇒ 4.5μg total)

---

The DNA was once again phenol extracted & EtOH ppt
— each 1&2 was redissolved in 20μl TE

17 was dated on file & counted





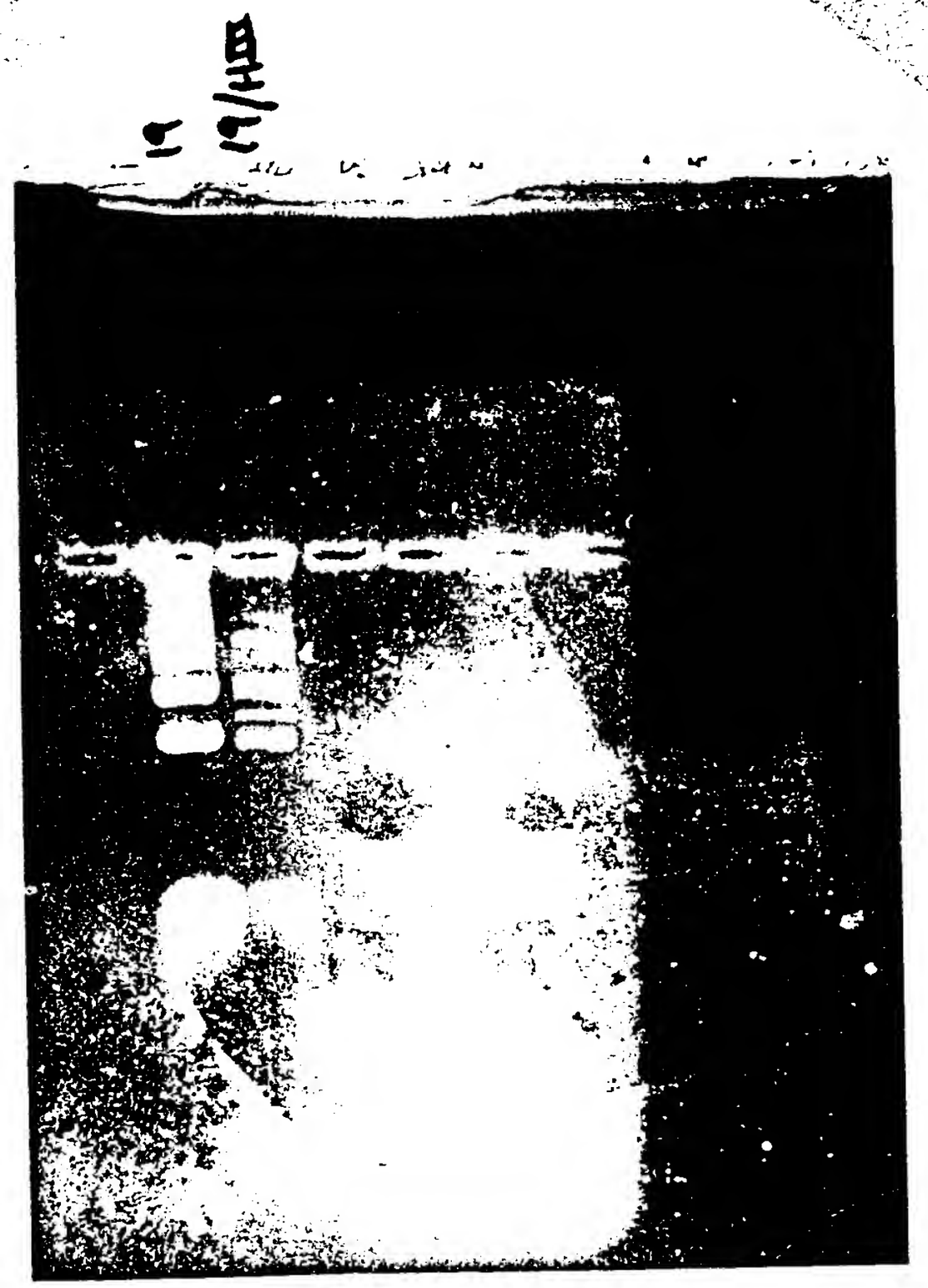
New Puc 19

~~Hpa I Puc 19 @ 13.07 ng/µl~~

~~Sac I Puc 19 @ 13.07 ng/µl~~

26.7 λ Puc 19 DNA @ 13.07 ng/µl (= 349 µg)
100 λ Hind III @ 10 U/λ (= 1,000 units)
14 λ 10x cut buffer
140.7 λ total

37°C @ 9:30 pm



← after 12 hours

To the above rxn mix:

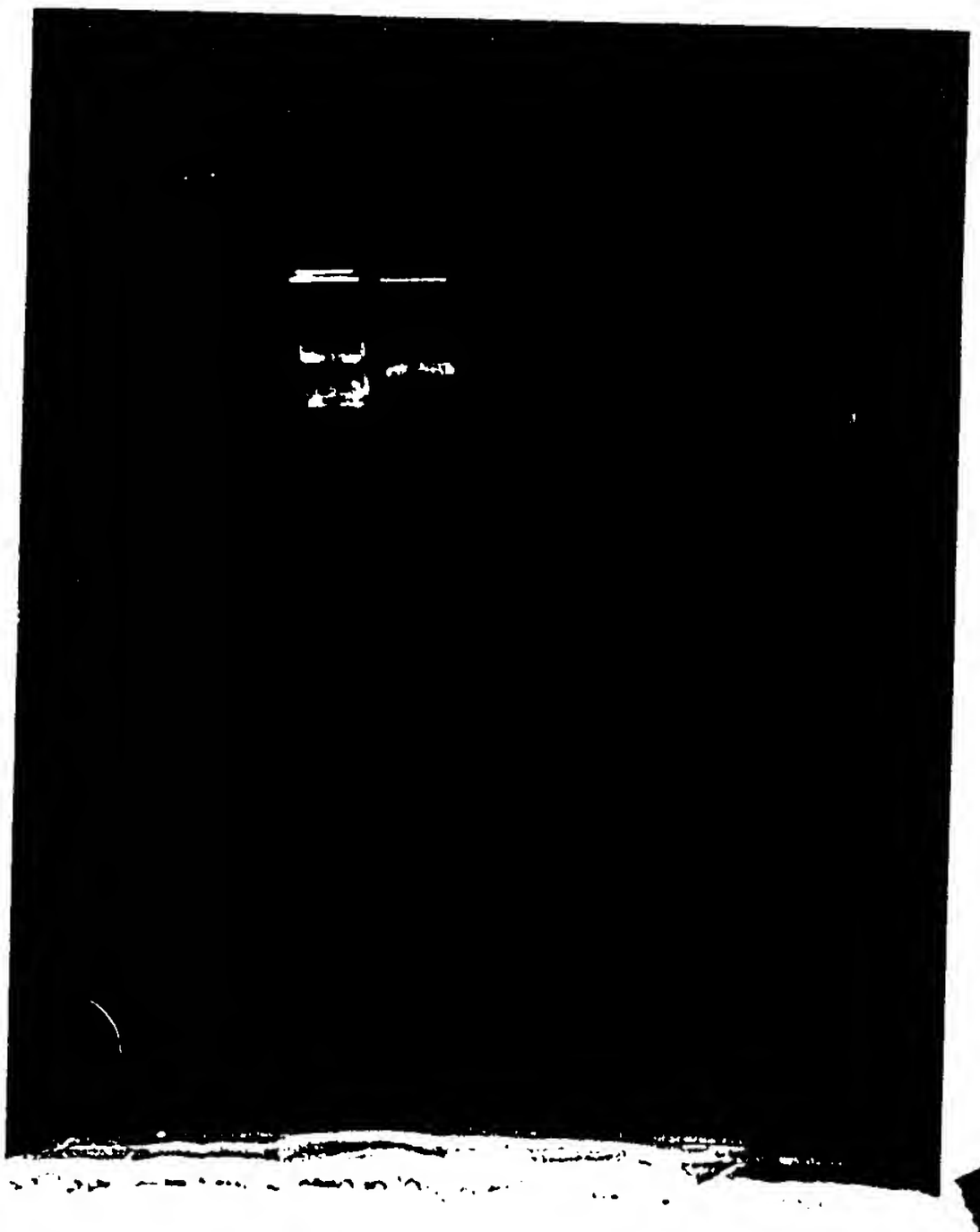
existing
- 26.7 PvuII @ 13.02 mg/ml (349 μg)
- 100 λ HindIII at 10 u/λ (1,000 units)
- 14 λ 10× core buffer
- 140.7 λ total

- 150 λ $H_2O$
- 50 λ HindIII
- 23.9 λ 10× core buffer

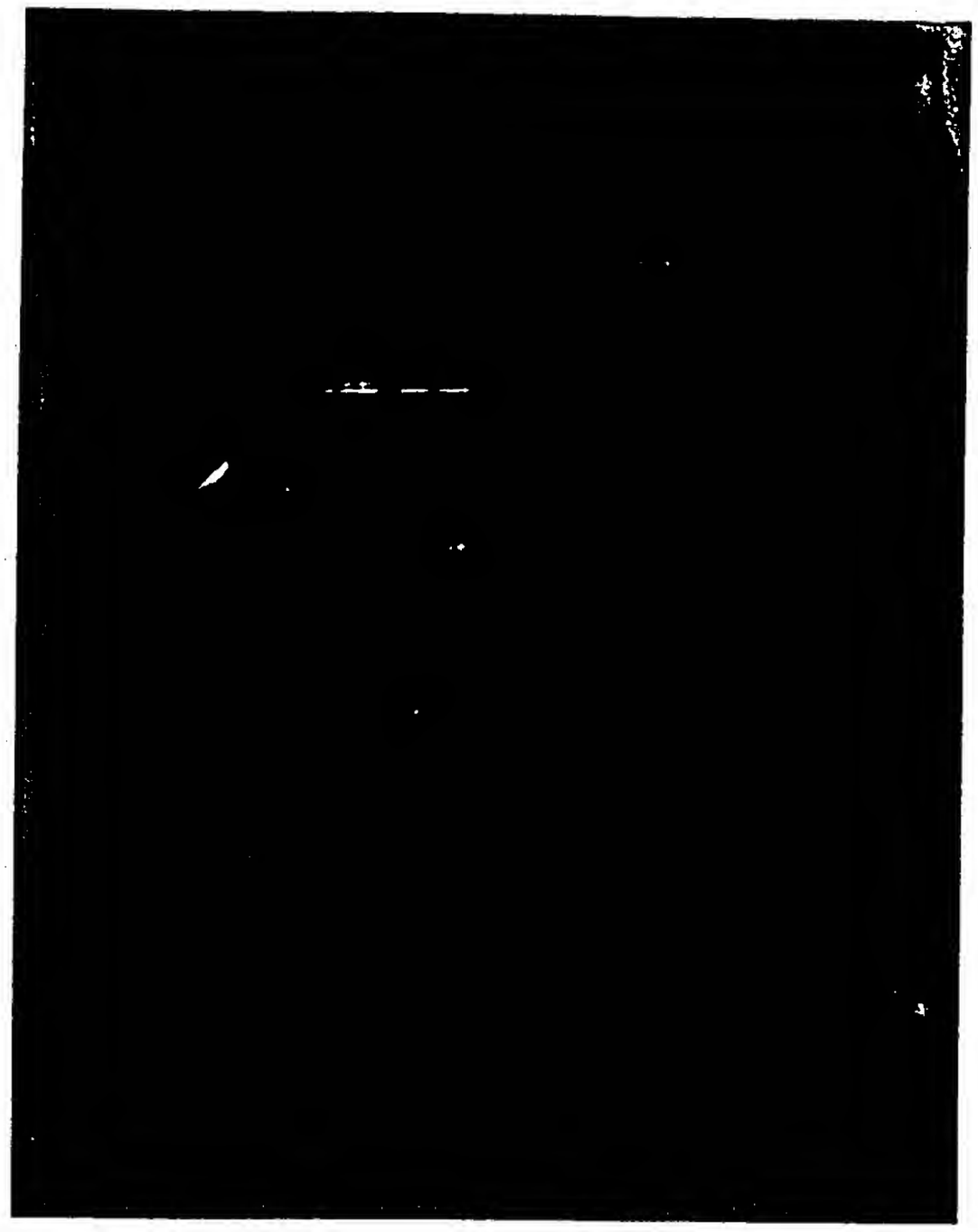
new total 364.6 λ
+180 λ $H_2O$
20 λ 10× core

364.6 λ total @ 11 am 7-25-84

Shows following dilution & addition of core buffer



Dec
Quasar



15λ of Lyman (reconst.) HII was added to Quasar mix (15λ · 94/λ) = 1354

2

Placed into 2 tubes

| | tube 1 | tube 2 | tube 3 |
|---|---|---|---|
| ~2401 | ~~2000~~ | ~~2000~~ ~2401 | 17-21 |
| | (extracted) | (extracted) | extracted |

~~Control~~ Control pt (1) OOH 1. OH/CHCl3 3. CHCl3
(extracted 4 times and combined aqueous phase)

The mixture was OOH, OH/CHCl3, and CHCl3 extracted but not OOH ppt'd. The organic phase was ~~discarded~~ pooled and "back extracted"

19/11 III

| nm | | "back extracted" |
|---|---|---|
| 260 | .500 | .305 |
| 280 | .278 | .197 |
| 320 | .001 | .001 |

diluted 1:40
↓↓
40X / 22

Concentration $\frac{40(.5)}{22} =$ ~~[illegible]~~

$= \boxed{.91 \mu g/\lambda}$

(~200λ ⇒ 182μg)

$\frac{260}{280}$ ratio 1.8

$\frac{40(.305)}{22} = \boxed{.55 \mu g/\lambda}$

(~220λ ⇒ 121μg

1.5

# TdT Assay

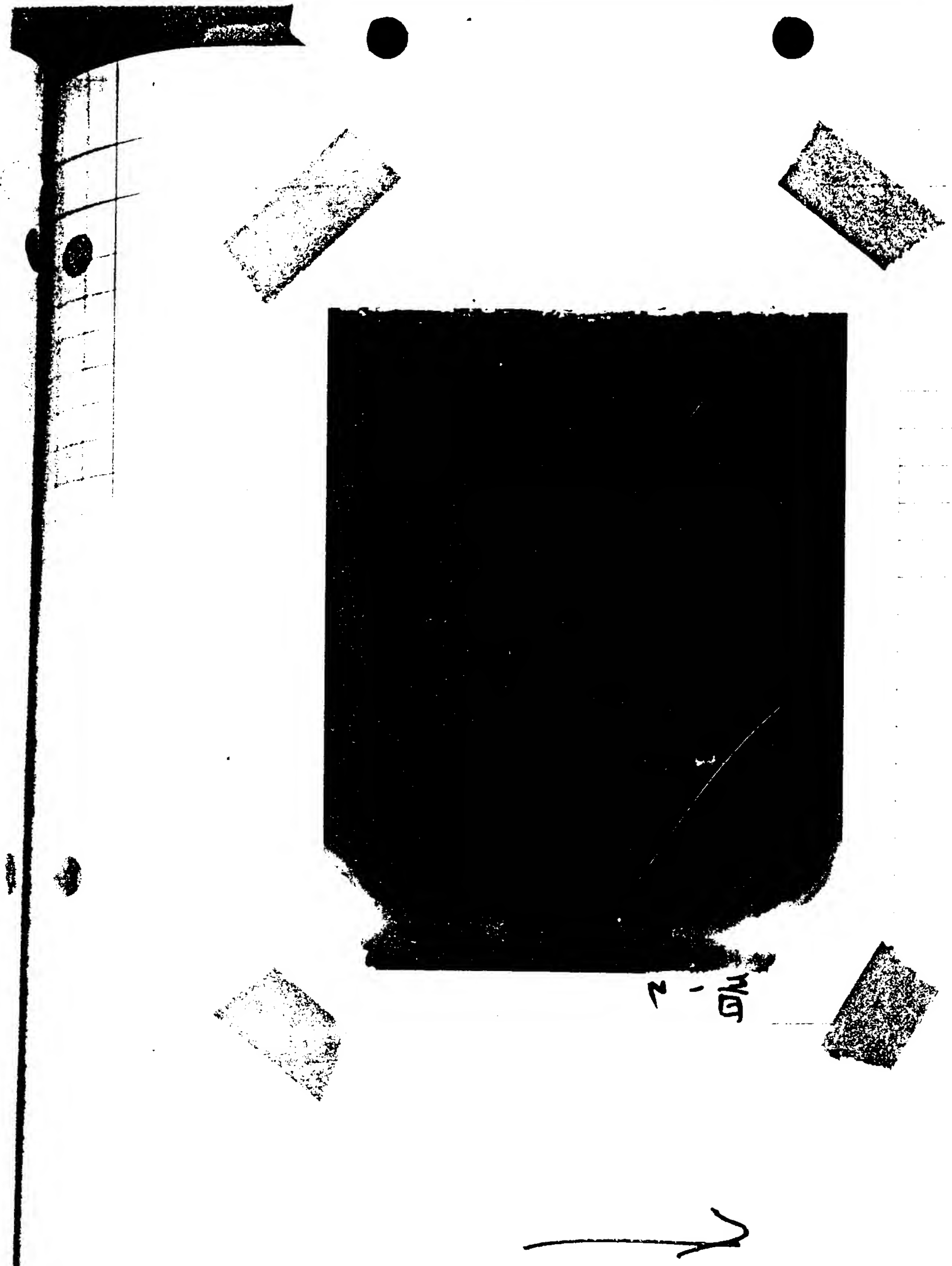
Goal: To check activity of P-L TdT
(based on assay on p. 41 this book)

✓ 5λ pool++ (5X) stock
✓ 0.8λ $^{32}$P dUTP 19 Ci/mM @ .9 μg/λ .72 μg
2.5λ P-L TdT
1λ $^{32}$P dTTP
2λ $^{3}$H dGTP
✓ 13.7λ $H_2O$
25 λ TdT cocktail

Same as above except
1λ $^{32}$P dCTP
2λ $^{3}$H dATP

Before adding TdT 2λ from each is spotted
After rxn 6λ from each is withdrawn & placed on a gel

051



T=000.50 A=07740.0(1.5%) B=01570.0(3.0%) C=107
T=000.50 A=017342.0(2.0%) B=054534.0(1.5%) C=090 436.0(1.0%) R=0.205
T=000.50 A=199944.0(0.7%) B=050312.0(1.5%) C=31 25?.0(1.0%) ?=3.144
T=000.50 A=092356.0(1.0%) B=257644.0(0.7%) C=45 612.0(0.7%) R=0.251
130.0(0.5%) S=0.25

4

$$\frac{17,342\ (2)}{92,356\ (17)}\ (1931) = 43$$

C $\frac{54,534}{257,644(17)}\ \frac{(2)}{}\ (6,435) = 160$

6 $\frac{73,740(2)}{199,944(47)}\ (8,336) = 362$

T $\frac{15,170(2)}{50,312(17)}\ (20,592) = 730$

| | # | % | |
|---|---|---|---|
| A | 43 | 3.3% | ⇒ 1.87 |
| C | 160 | 12.4% | |
| 6 | 362 | 28.0% | |
| T | 73? | <7.4 | |

. quit A = .033 C = .123 G = .28 T = .564
*** END RUN dBASE II ***

```
term =  1.1036923-02
ala  =  .03444
arg  =  3.7332125-02
asn  =  7.4314335-04
asp  =  6.3478835-03
cys  =  .1084911
gln  =  1.2704677-03
glu  =  2.0921275-0
gly  =  .0784
his  =  2.7865335-03
ile  =  1.3400647-02
leu  =  .1689361
lys  =  3.4085735-04
met  =  5.2113617-02
phe  =  .218532
pro  =  .015123
ser  =  7.5719035-02
thr  =  .0304059
trp  =  .0442171
tyr  =  1.2706447-0 1
val  =  .15792
```

A new batch of pool++ was prepared according to recipe on p.32 in

TdT Assay: To test out new pool in anticipation of yet another large scale tailing rxn

| | | |
|---|---|---|
| ① 5λ | pool++ (5X) | Std |
| 0.8λ | Puc 19/HindIII @ .91 μg/λ | .72 μg |
| 1.8λ | P-L TdT | |
| 1λ | α-32 PdTTP | |
| 2λ | 3H dGTP | |
| 13.2λ | $H_2O$ | |
| 25λ | total | |

② as above except
1λ α-32PdGTP
2λ 3H dATP

rxn went 45 min.

Before adding TdT spot 2λ

After rxn 5λ withdrawn for gel

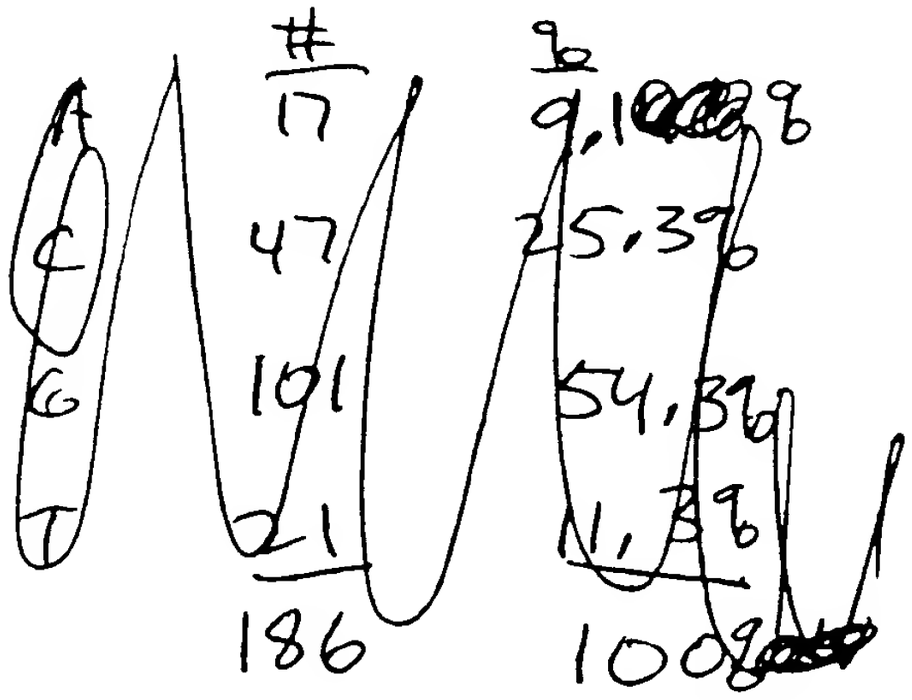
Run out on agel - looked as expected; not photographed

(A) $\frac{11,614\ (2)}{152,126\ (17)}\ (1931) = 17$

(C) $\frac{13,044\ (2)}{211,308\ (17)}\ (6,435) = 47$

(G) $\frac{22346\ (2)}{217,204\ (17)}\ (8,336) = 101$

(T) $\frac{1,868\ (2)}{46,506\ (17)}\ (20,592) = 97.3$




| | # | % |
|---|---|---|
| A | 17 | 6.5% |
| C | 47 | 17.9% |
| G | 101 | 38.5% |
| T | 97.3 | 37.1% |
| | 262 | 100% |

TdT Assay

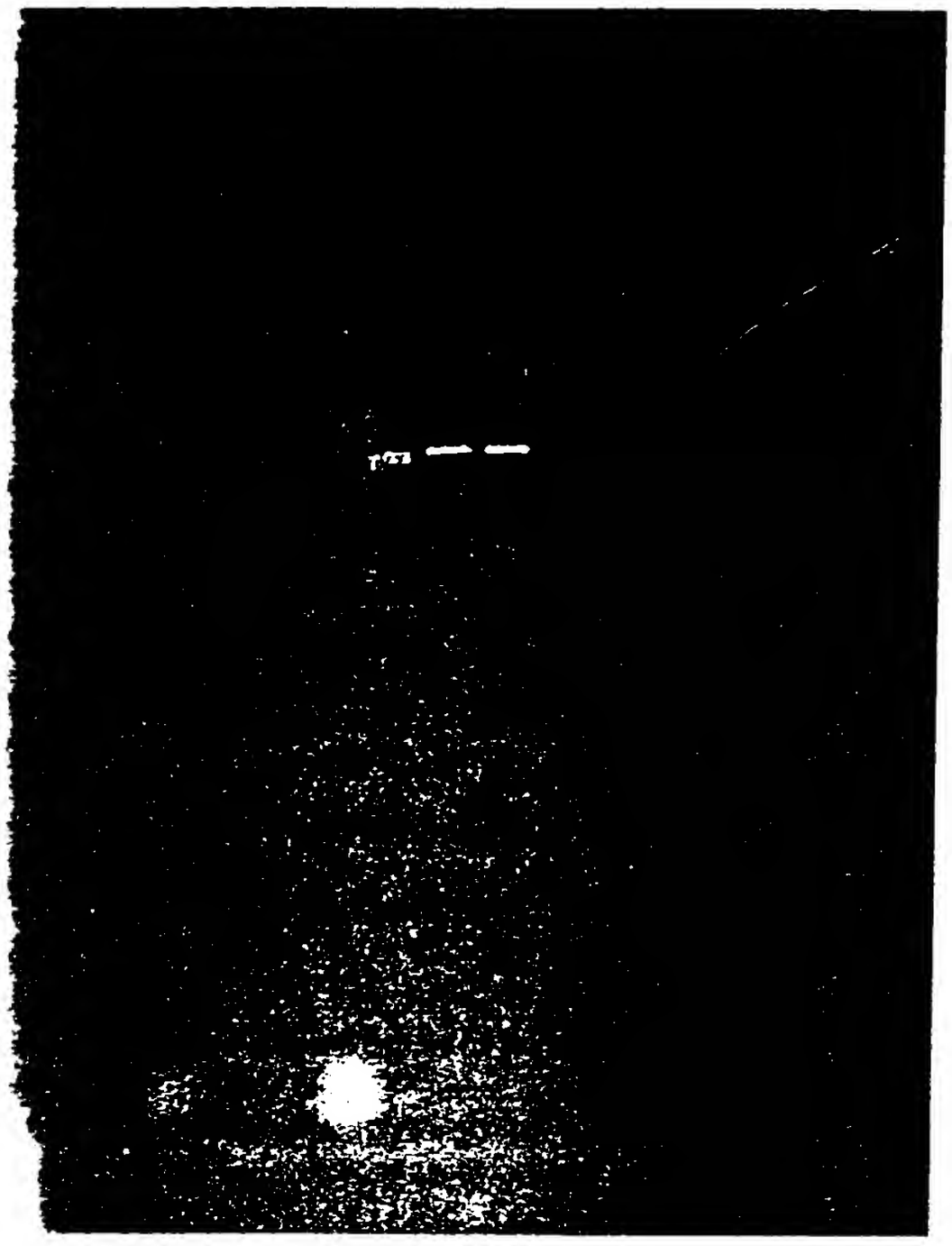
This assay uses the old "batch 2" of TdT (see p. 3W)

1. 5λ pool ++ (5X) — std
   0.8λ pUC19/HIII @ .91μg/λ — .72μg
   1.5λ "batch 2" TdT
   1λ α-32 PdTTP
   2λ 3H dGTP
   14.7λ $H_2O$
   25λ total

2. Same as above except
   1λ α-32P dATP
   2λ 3H dATP

- Before adding TdT spot 2λ
- After 1 hr incubates at room temp for gel check

~~1 hour~~ 45 min ea.



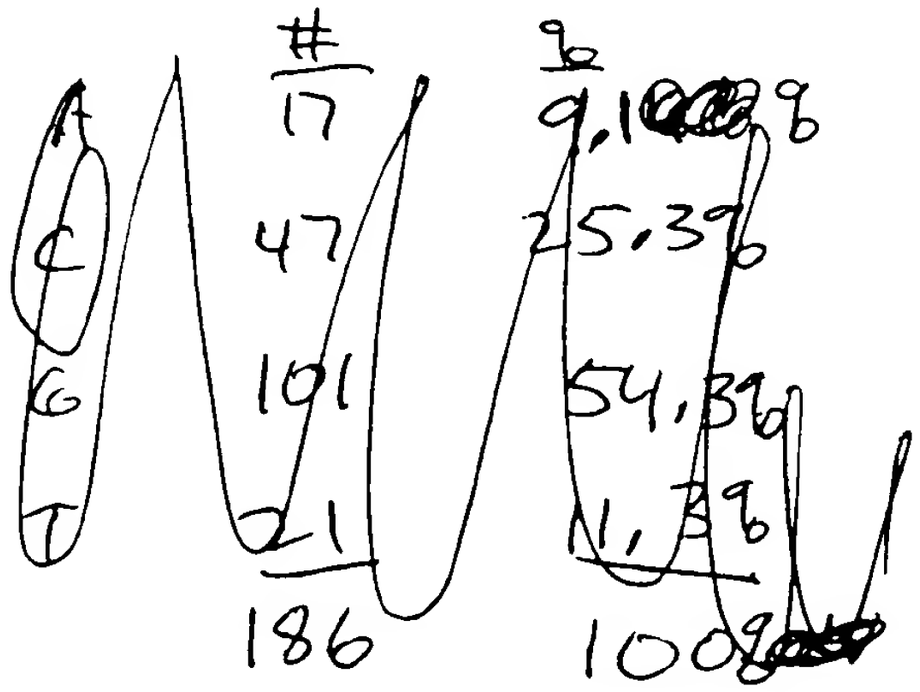
T=000.50 A=022346.0(2.0%) B=001368.0(2.0%) C=028900.0(2.0%) D=0.033
T=000.50 A=011614.0(3.0%) B=013044.0(1.0%) C=030044.0(2.0%) D=0.184
T=000.50 A=217204.0(0.7%) B=046506.0(1.5%) C=322304.0(0.5%) D=0.213
T=000.50 A=152126.0(1.0%) B=211308.0(0.7%) C=450404.0(0.5%) S=0.213

(A) $\frac{11,614\ (2)}{152,126\ (17)}\ (1931) = 17$

(C) $\frac{13,044\ (2)}{211,308\ (17)}\ (6,435) = 47$

(G) $\frac{22346\ (2)}{217,204\ (17)}\ (8,336) = 101$

(T) $\frac{1,868\ (2)}{46,506\ (17)}\ (20,592) = 97.3$




| | # | % |
|---|---|---|
| A | 17 | 6.5% |
| C | 47 | 17.9% |
| G | 101 | 38.5% |
| T | 97.3 | 37.1% |
| | 262 | 100% |

TdT Assay: To find the proper amt of batch 2

| | |
|---|---|
| 1.5λ pool ++ (5x-batch 2) | std |
| 0.8λ pUC19/HindIII @ .91 µg/λ | .72 µg |
| 6.5λ "batch 2" TdT | |
| 1λ $\alpha$-32 PdTTP | |
| 2λ $^3$H dGTP | |
| 9.7λ $H_2O$ | |
| 25λ total | |

2. Same as above except

1λ $\alpha$-32 PdCTP

2λ $^3$H dATP

- before adding TdT spot 2λ

~~After RXN complete remove 5λ for gel check~~

~~1 hr~~ 40 minutes

001 T=000.50 A=008006.0(3.0%) B=001486.0(1.0%) C=01(552.0(3.0%) R=0.185
002 T=000.60 A=009356.0(3.0%) B=012738.0(0.0%) C=01(588.0(3.0%) R=1.297
003 T=000.50 A=120616.0(1.0%) B=049258.0(1.5%) C=17.0078.0(0.7%) R=0.365
004 T=000.50 A=174738.0(0.2%) B=284746.0(0.7%) C=320492.0(0.5%) S=0.3[illegible]

[A] 9356 / 174,738 (11.5) (1931) = 9

[C] 12138 / 284,746 (11.5) (6435) = 24

[G] 8006 / 120,616 (11.5) (8336) = 48

[T] 1486 / 44,258 (11.5) (20,592) = 60

| | # | % |
|---|---|---|
| A | 9 | 6.4% |
| C | 24 | 17.0% |
| G | 48 | 34% |
| T | 60 / 141 | 42.6% / 100% |

There appeared to be a lot of glycerol in rxn mixture — maybe so much so that the rxn was inhibited

# TdT Assay

1. ✓ 5λ pool++ (batch 2)
✓ 0.8λ pUC19/H$^{III}$ @ .91 μg/λ .728 μg DNA
3λ "batch 2" TdT
1λ $\alpha$-$^{32}$PdTTP
2λ $^{3}$HdGTP
✓ ~~0~~ 13.2λ $H_2O$
25λ total
→ actually 23λ total since 2λ withdrawn

2. Same as above except
1λ $\alpha$-$^{32}$PdCTP
2λ $^{3}$HdATP
45'

Before Adding TdT withdrawn 2λ
3λ withdrawn for gel
→ checked, not photographed — latter good — no detectable exo activity

~~#3 of~~ The calculations of the length is WRONG. It depends on the concentration of DNA ~~that~~ originally in the original ~~assay~~ since part of it, however, more DNA was added. Revised # ~~of~~ of NTPs/assay are below.

(See p. 32 yellow)

~~5 mg/ml~~

~~$\Rightarrow$ (.05 l) (1.15 x 10^12 molecules/assay per ml)~~

$5\ mg \Rightarrow 55(1.15\times10^{-12}\ \frac{3'OH\ Molecules}{\mu g})$

$= 6.325\times10^{-11}$ molecules 3'ends

$$\frac{6.325\times10^{-11}\ \text{molecules 3'OH}}{.0010725\ l}$$

$= 5.9\times10^{-8}\ M$

$.06mM = .06\times10^{-3}\ M$

$= 6\times10^{-5}\ M$

#A $\frac{6\times10^{-5}\ M\ dATP}{5.9\times10^{-8}\ M\ 3'OH} = 1,017$

#C $\frac{2\times10^{-4}\ M}{5.9\times10^{-8}} = 3,390$

~~2783 tdTs~~

# TdT Radiation Tailing - Large Scale

~~[illegible] λ pUC19/HIII @ .9/μg/λ~~
~~[illegible] pdTTP~~
~~[illegible] Buffer [illegible]~~

~~42λ pUC19/HIII @ .9/μg/λ~~ ~~110μg DNA~~

429 fold scaleup

1. ~~60.5λ~~ pUC19/HIII @ .9/μg/λ 55μg DNA
   ~~215λ~~ pdTTP
   129λ TdT - "batch 2" (@ 100μg/ml)
   6λ $\alpha$-32PdCTP
   10λ $^3$HdGTP
   ~~652λ~~ $H_2O$
   1072.5 total

2. Same as above except
   6λ $\alpha$-32PdCTP
   10λ $^3$HdATP

- Before adding TdT withdraw 5λ and spot from each tube
- At conclusion of rxn withdraw from each tube
  - 10λ for counting - into culture tube
  - 6λ for mini gel

~~410 units~~

Note [illegible] was withdrawn before the [illegible] quantity of TdT [illegible] added ⇒

$\frac{107.5 - x}{107.5} = \frac{}{}$ =

$\frac{107.5}{107.5 - 7.5}$ = 1.14 X as concentrated

[illegible] 5 ml (1.14) = 5.68 ml of pool equiv. drawn

T=000.50 A=03318.0(5.0%) B=00202.0(.20%) C=00410.0(5.0%) T=0.99
T=000.50 A=01248.0(.19%) B=02003.0(2.0%) C=02135.0(1.8%) R=1.518
T=000.50 A=02962.0(1.0%) B=23252.0(2.0%) C=11308.0(1.0%) R=0.381
T=000.50 A=36228.0(2.0%) B=76976.0(1.5%) C=74308.0(1.5%) S=0.981

[A] $\frac{1248\ (5.68)}{36228\ (10)}(1931) = 38$ .0195

[C] $\frac{2008\ (5.68)}{76,976\ (10)}(6,435) = 95$ .0148

[G] $\frac{3318\ (5.68)}{82962\ (10)}(8,336) = 189$ .0227

[T] $\frac{302\ (5.68)}{23352\ (10)}(20,592) = 151$ .00735

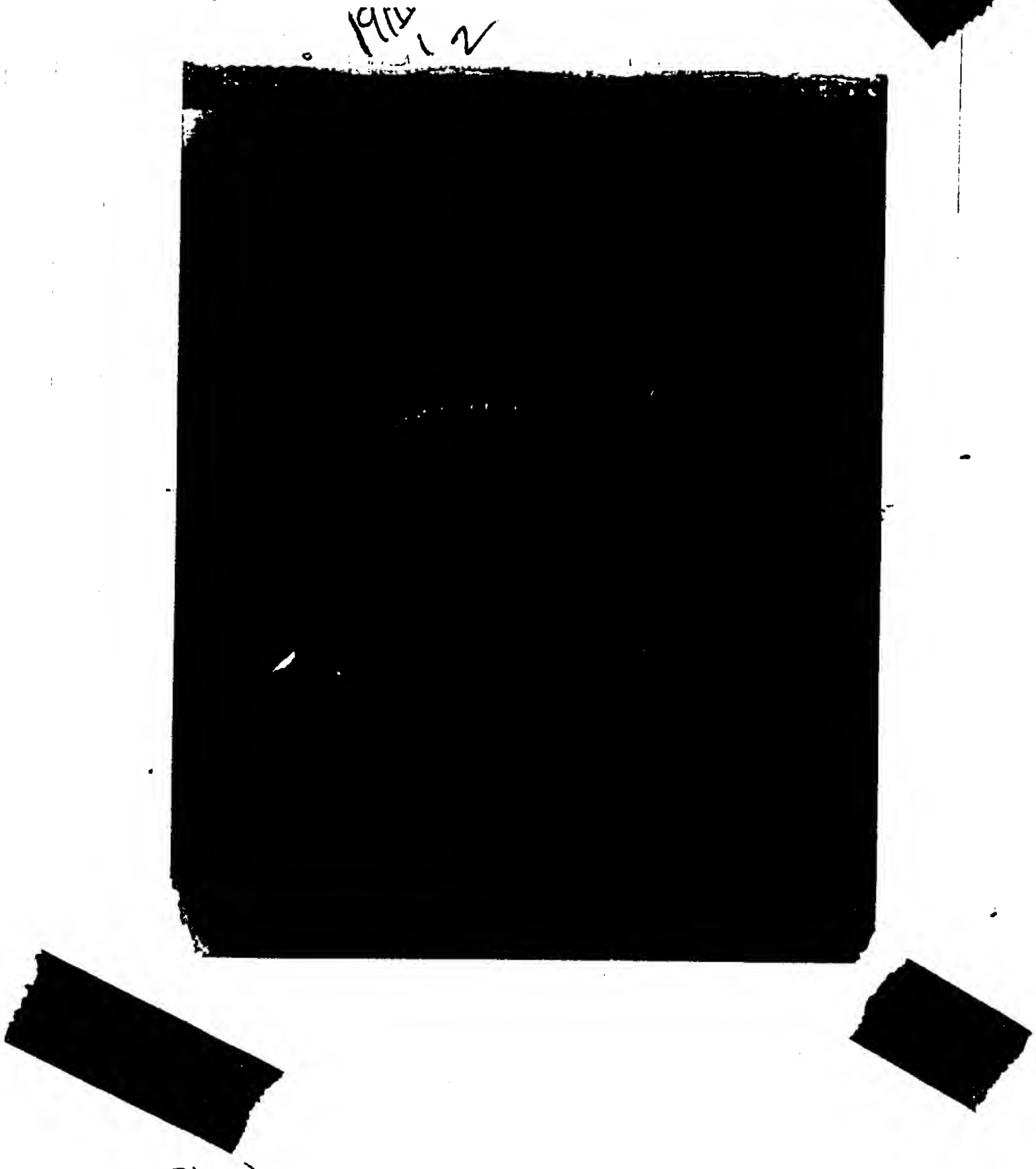
| | | |
|---|---|---|
| A | 38 | 8.0% |
| C | 95 | 20.0% |
| G | 189 | 40.0% |
| T | 151 | 32.0% |
| | 473 | 100 |

249

1-87

P(TAA) (.08)(.32)(.32) = .00819
P(TAG)
P(TGA) )) = (.32)(.08)(.40)
= .01024
= .99432

100 ⇒ 15.6%
(98.1568)



I'm somewhat skeptical of the counting results - the gel shows a definite smeary insert

Sfug
[redacted] extracted
LL phenol, 1x LL phenol/chloroform
chloroform → ether

#1 phenol'd & checked on a gel - looked good
So #2 also phenol'd, but not checked
both #1 & #2 need to let ether further
evaporate from them

---

~1/2 of #1 loaded into Nacs
(~27.5 µg DNA)
→ 520 µl diluted to 5.2 ml w/ binding buffer

eluted in ~~250 µl 3x250 µl~~
825 µl 3x275

EtOH ppt

pellet size recovered quite small

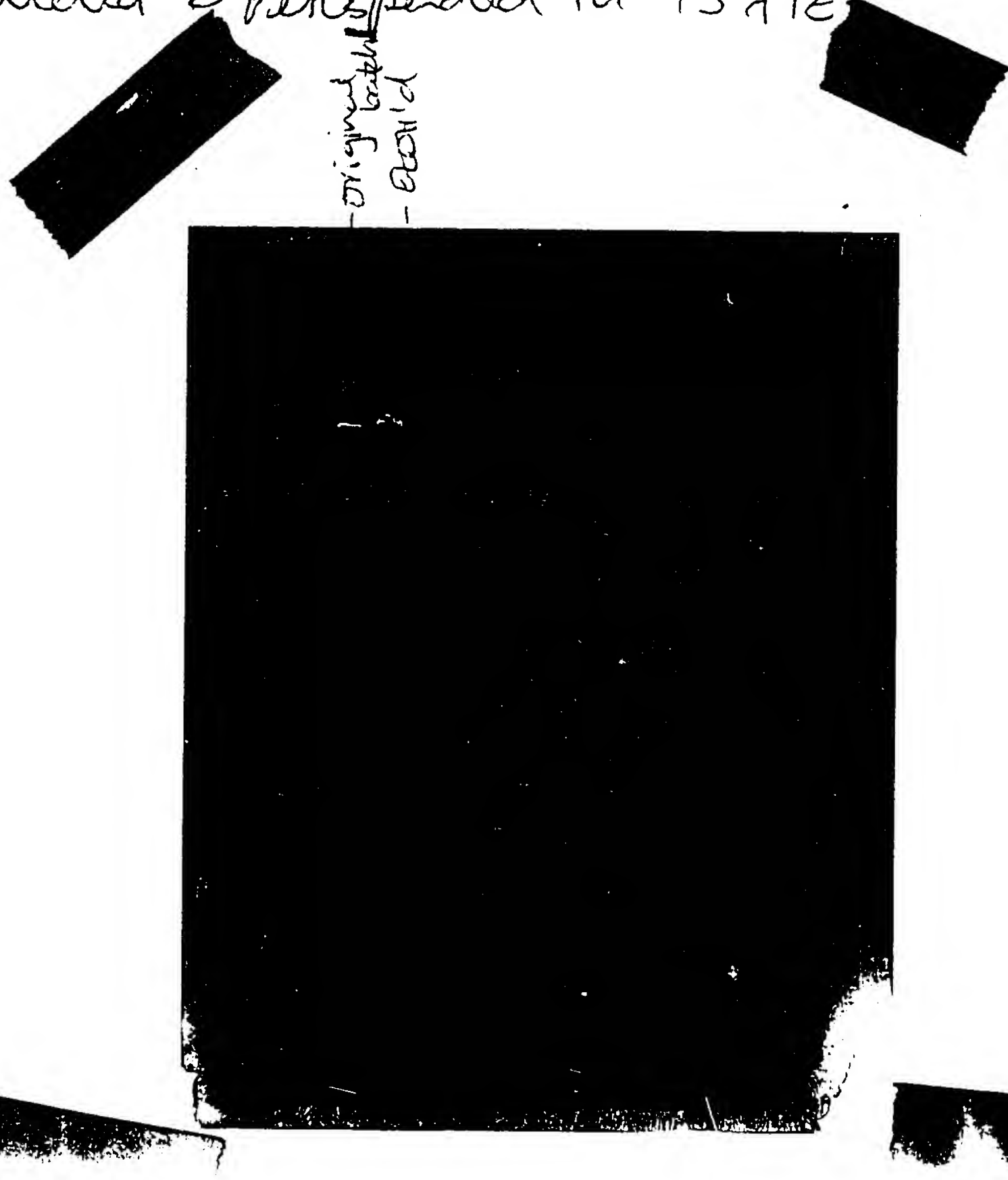
Attempted Purification scheme: EtOH ppt — but first chelate w/ EDTA

100λ of rxn #1
5.5λ of 500mM EDTA
11λ 3M ~~Sodium~~ acetate
291λ 95% EtOH

5μg DNA in 1mM $CoCl_2$
20mM EDTA
283mM $NH_4$ acetate

* Note: Not $NH_4$!

pelleted & resuspended in 15μTE



00 measurements

| | raw r&n #1 (phenol'd) | EtOH'd #1 |
|---|---|---|
| 260 | .439 | .738 |
| 280 | .279 | .513 |
| 320 | ~~0~~ | .057 |

1:40 dilution

Counting Experiment

"P1" = 3λ of #1 raw and 3λ of #1 EtOH'd were dropped on filters dryed & counted

"P1E" =

"1" = 3λ of #1 raw and 3λ of #1 EtOH'd were acid ppt and filtered & counted according to the normal polymerase assay

"1E" =

I refers to rxn mixture #1

32P adapt. A: 3H B: 32P

[illegible] T=000.50 A=0[illegible]6.0(1.5%) B=0[illegible]2.0(3.0%) C=05[illegible]0.0(2.5%) R=0.015

[illegible] T=000.50 A=1[illegible]5.0(1.0%) B=021[illegible].0(2.0%) C=17[illegible]4.0(0.7%) R=0.140

[illegible] T=000.50 A=0010[illegible].0(.10%) B=000[illegible].0(.10%) C=00142.0(1.5%) R=0.055

[illegible] T=000.50 A=00434.0(5.0%) B=000[illegible].0(.30%) C=01[illegible]04.0(0.5%) S=0.005

acid ppt: 32P

(these vertical lines are here by mistake)

Interpretation & calculations from the above

I. First of all, these results (#1 & 1 should be in agreement with the earlier pool & acid ppt counts

A. P.58W ⇒

P1: $\frac{3}{5}(82,962) \stackrel{?}{=} 44,566$ ⇒ Counting of pool for rxn #1 good

$^3H$ 49,777 ≈ 44,566

B.

$^{32}P$ $\frac{3}{5}(23,352) \stackrel{?}{=} (10,928)$

14,011 ≈ 10,928

B.

1: $^3H$ .3(3318) $\stackrel{?}{=}$ 1090

995 ≈ 1090 ⇒ Counting of acid ppt'ble counts for rxn #1 good

$^{32}P$ .3(302) $\stackrel{?}{=}$ 66

90.6 ≈ 66

III. Recovery on EtOH ppt

(Note that phenol extraction is assumed to have 100% - this should be traced)

For "1" 3λ of the crude mixture had 1090 3H
for "1E" 3λ had 4644 3H counts

but 1E represents 100λ of "1" resuspended
in only 15λ TE

$\frac{100}{15}$ = 6.7 fold concentration

$\frac{4644}{1090(6.7)}$ = 64% recovery

Based on 32P counts

$\frac{328}{66(6.7)}$ = 74% recovery

⇒ ~70% recovery of DNA

IV. % dNTP removed by EtOH ppt:

3H { counts due to dNTP alone
~~49,566(6.7) - 1090(6.7)~~ ← before EtOH ppt
133,176 - 4644 ← after EtOH ppt

3H { counts due to dNTP alone ← after EtOH ppt
$\frac{133,176 - 4644}{(\text{illegible})}$ = .44 % all dNTP due to dNTP remaining

32p ~~21394-328~~

$$32p \left\{ \frac{21394-328}{(10,928-66)(6.7)} = .29 \right.$$

3H ⇒ 56% of unincorporated dNTP removed

32p ⇒ ~~@~~ 71% of unincorporated dNTP removed

Conclusions-

1. ~70% of the DNA was recovered by EtOH ppt. (concentration of DNA computable from this fact)
2. ~60% of the unincorporated dNTP was removed
3. A relatively small percentage of the counts even following EtOH ppt are due to the DNA. Despite removal of over half of the unincorporated dNTP only ~1.5% of the total counts are acid ppTable
4. When EDTA was added & the ~~[illegible]~~ switched to Na acetate (in place of ammonium acetate) degradation of the DNA did not occur
5. Perhaps the Nitcs purified stuff—although very cold—is just pure DNA. The pellet was not very big, but counting it should tell for sure after all EtOH ppt of

Ethanol ppt of the remaining RNA mix

#1 [illegible] = 453λ

#2 [illegible]

#1

453λ of #1 [illegible] (23μg of DNA)
25λ of 300mM [illegible]
250λ 3M Na [illegible]

350λ of #1 [illegible] 18μg of DNA
19.3λ of [illegible]
397λ of 3M Na [illegible]
517λ EtOH
1225λ total

50λ of #1 [illegible]
3.3λ of [illegible]
16.5λ 3M Na [illegible]
350λ EtOH
425λ

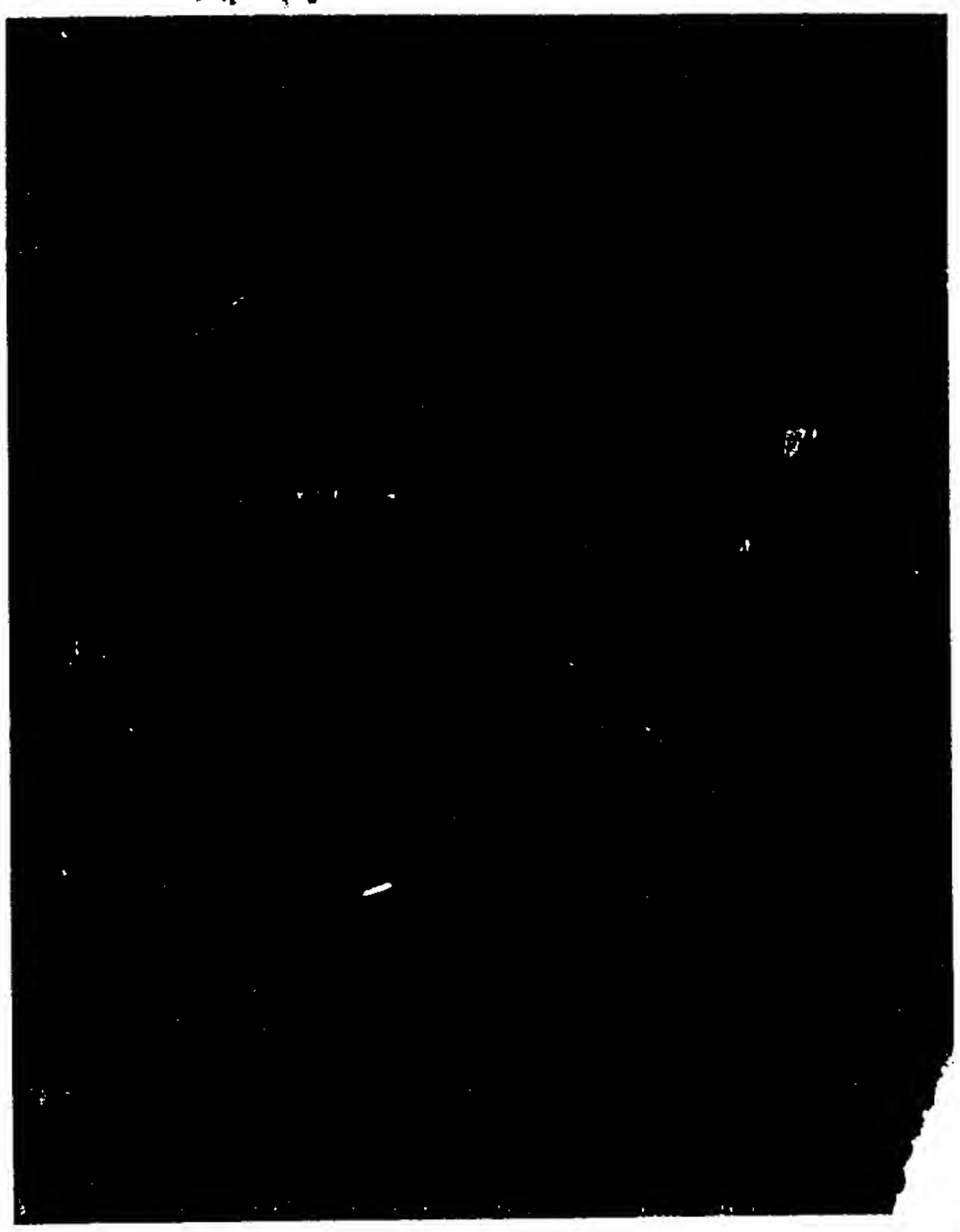
#2 - [illegible] to get reference [illegible]
divide [illegible] aliquots of 350λ and treat as #1

#1 [illegible] DATE
#2 [illegible] SCAN TE

2λ [illegible]

[illegible] IEA ⟷ [illegible] ppt as opposed to [illegible] spin

2- Ethanol ppt
1- Ethanol ppt - large bubbles



This gel shows that the DNA has maintained it's integrity following each ppt.

2% of each counted

from recounting = 21 cpm

Subsequent recounts

2% each counted

I. Do these results give earlier pool & acid precipitable count data?

A. p. 58W ⇒ Check of "pool" results

$^{3}H$ { $\frac{2}{5}(36{,}228) \doteq 10{,}080$

14,491 ≈ 10,080

⇒ fairly consistent agreement

$^{32}P$ { $\frac{2}{5}(76{,}976) \doteq 20{,}348$

30790 ≈ 20,348

The $^{3}H$ data should be more accurate for this comparison because the $^{32}P$ is in continuous decay.

B. Check of acid ppt'able count results

$^{3}H$ { .2(1248) ≐ 254

250 ≈ 254

⇒ fairly consistent agreement

$^{32}P$ { .2(2008) ≐ 254

402 ≉ 254

II, Recovery of Etoh ppt

A. Rxn #1

$^{3H}$ { [redacted] 3052(1.5) / 1090(17.5) — [redacted] 24% recovery

27λ → 3λ

1EA 350λ represents 350λ of 1A resuspended in only 20λ ⇒ $\frac{350}{20}$ = 17.5X concentration upon resuspending → 21

$^{32P}$ { [redacted] 2578(6.5) / 66(17.5) = [redacted] 27% recovery

3,350 [redacted] recovery

Clearly, something [redacted] is wrong as $^{3H}$ & $^{32P}$ don't jive

.25(18μg) / 20λ = .23μg/λ → 20λ → 4.5μg

B. Rxn #2

4131 → upon resuspending

$^{3H}$ { 6944 / 254(20) = [redacted] recovery / 81% recovery

#2 was ~1000λ redissolved in 50λ ⇒ 20 fold dilution

$^{32P}$ { 2526 / 254(20) = 50% recovery

⇒ on the average ~ [redacted] 65% recovery

⇒ .65(55μg) / 50λ = .72μg/λ

50λ = 36μg of DNA

III. Removal of dNTP

A. rxn #2

~~10,080 - 254~~
~~37,992 - 6,794~~

$$^{3H}\left\{\frac{37,992-6,794}{(10,080-254)20}\right. = .16$$

⇒ 84% of all unincorp dNTP's removed

$$^{32P}\left\{\frac{147,462-2,526}{(20,348-254)(20)}\right. = .36$$

⇒ 64% of all unincorp dNTP's removed

< ~75% of all unincorp. dNTP's removed

B. rxn #1

~~1090 - 66~~

$$^{3H}\left\{\frac{(103,540-3052)(1.5)}{(44,566-1090)(17.5)}\right. = .19$$

⇒ 81% of all unincorp dNTP's removed

$$^{32P}\left\{\frac{(17,252-2578)(1.5)}{(10,928-66)(17.5)}\right. = .12$$

⇒ 88% of all unincorp dNTP's removed

# Attempted Annealing, Fill & Ligation

## Anneal

| | | |
|---|---|---|
| 2.8λ | #2 rxn/phenol'd/EtOH'd @ .72μg/λ | 2μg DNA in 20mM Tris |
| .8λ | 1M tris pH 7.5 | |
| 36.4λ | $H_2O$ | |
| 40λ | total | |

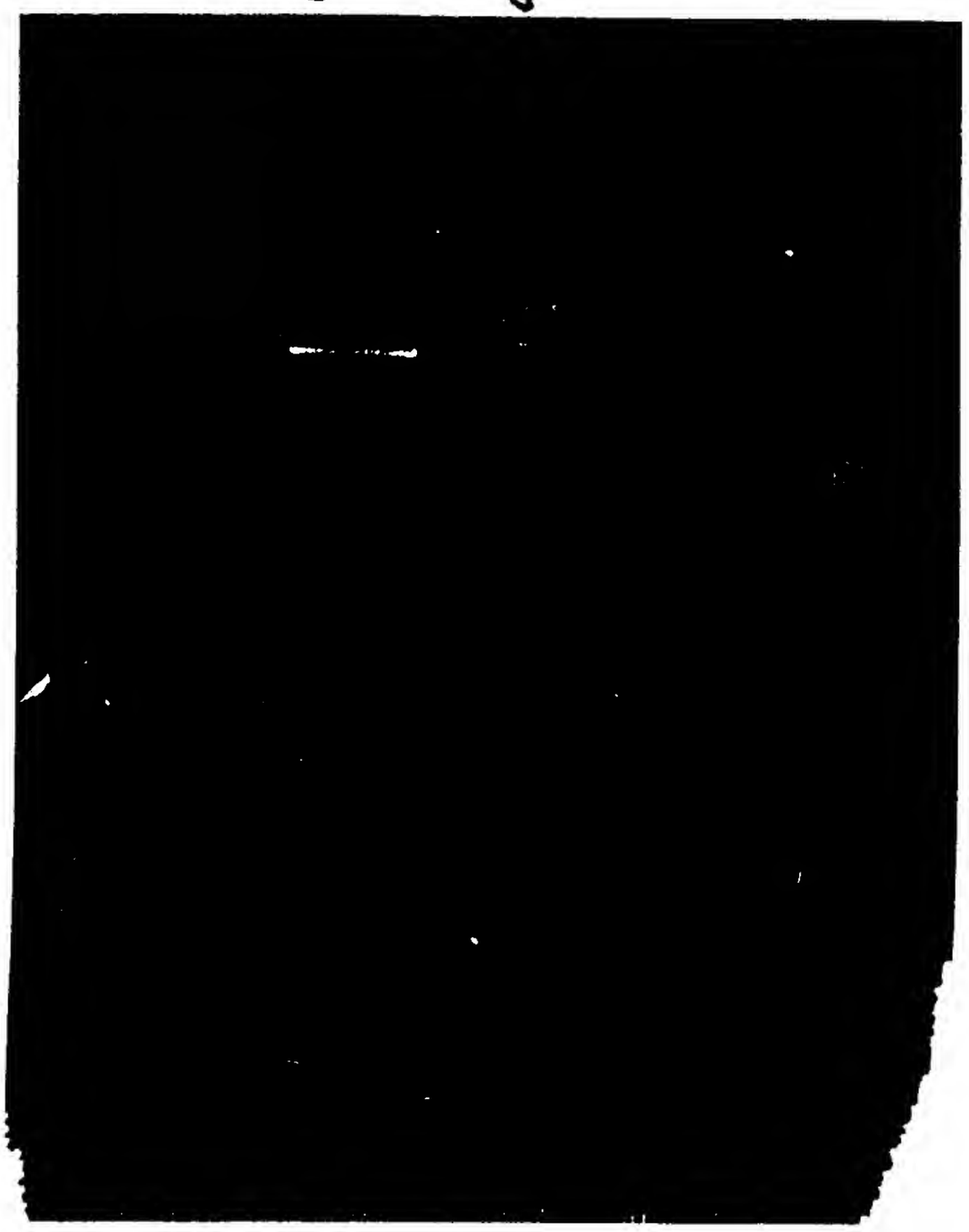
1. heat to 68°C for 5 minutes
2. incubate at 58°C for 1½ hours

## Fill-in & ligation

| | | |
|---|---|---|
| 40λ | of #2 rxn/p'd/e'd DNA at 50ng/λ (from above) (=2μg) | 333ng/λ |
| 12λ | of 54λ Klenow Pol I from BRL (4 units) | 1U/λ |
| 1.2λ | .5M dTT | 10mM |
| 0.6λ | .1M ATP | 1mM |
| 0.6λ | 1M $MgCl_2$ | 10mM |
| 3λ | of 24λ T4 DNA ligase (=6 units) (BRL Lyophilizate) | .1U/λ |
| 0.5λ | 5mM dATP | 42μM |
| 0.5λ | 5mM dCTP | 42μM |
| 0.5λ | 5mM dGTP | 42μM |
| 0.5λ | 5mM dTTP | 42μM |
| 59.4λ | total | |

3:20pm Avg. 15
incubated at room temp = 22.2°C

23.8




Is it circular or linear — try restricting it! yes, but restriction sites will randomly appear also & then all event will

unique sites in PUC19 vector → NAE ECORV 1 1 6 1

with digested

~~(100 ng) Annealed mix (=267 ng DNA)~~

9:45 -- 1ml of overnight → 200ml x4T

The following plates were made

| PUC19 (unmodified; uncut) | The PUC19/random (from "annealing mix") |
|---|---|
| .5ng | .5ng |
| 5ng | 5ng |
| 50ng | 50ng |
| | 500ng |

The DNA was diluted accordingly

↳ incubated at 2:15pm

# Transformation Protocol

## $Ca^{++}$ Cells

1. Pick a single colony (or from stock glycerol) of JM83 and innoculate into a 20~~0~~ml 2xYT at 37°C in a shaker overnight. (Use a large flask ~~for good aeration~~ 125ml)

2. Take 1ml of this overnight culture and innoculate a 200ml 2xYT culture (a large flask again) for 2hr

While growing place a 250ml sorvall bottle at -20°C to cool

3. At $A_{600}$ = .6 or "just a little less" harvest culture by centrifugation at 5K for 8 minutes at 0-4°C.

4. Decant supernatant (clear and amber). Resuspend cell pellet in ice-cold 50mM $CaCl_2$ (filter sterile). Use 1/2 original grown volume (~100ml); resuspend by swirling; do not pipette to resuspend. Incubate on ice for 20 min. w/ occasional swirling

5. Repellet for 5K at 0-4°C for 8 min.

6. Decant supernatant (clear and colorless) and resuspend cells in 1/10 original volume (~20ml) of ice cold 50mM $CaCl_2$

7. Store cells on ice in cold room.

NB:
(i) keep everything cold! (on ice)

For mp selection: add
1 ml of 2XYT to each
tube and incubate in 37°C
water bath for 1 hour

## Transfection

1. For every desired plate, place 0.3ml Ca++ cells in a small culture tube and add DNA. Incubate 40 minutes on ice (20 minutes should be sufficient)

   Meanwhile, make top agar, 2ml per plate (2XYT heated in microwave with .9% agar; .09g/10ml don't let boil over in microwave; prepare in small conical culture flask) and keep warm on hot plate set at "4.2 and low" (=

2. After the 40 minutes is up heat shock the tubes for 2 min in a 42°C water bath (make this bath in a styrofoam tub w/ sink water, (Warm to room temp the plates to be used)

3. Remove from heat and add to the soft agar the following on a per tube amount

   per tube, but add to soft agar to mix!

   15λ IPTG (.1M)
   75λ X-Gal (=10, 1:10 in MeFo 50 dimethylformamide)
   6λ 3X amp @ =5mg/ml => 75μg/ml)

4. Add 2ml of the above mix per each tube of Ca++ cells; quickly vortex and pour on to 8 agar AMP plates; swirl to coat the whole plate.

5. Incubate upside down at 37° overnight

Results:

More-or-less a lawn on all plates ~~& control~~ even neg. control & AMP lanes

A few blue colonies on the periphery of the "19" plates ~~A 50~~ had 2 or 3 blue colonies on the periphery

Making more "anneal II":

- 2x the protocol on p. 65 was followed → incubation at 55° to 65°

- also "anneal control" at 1x the original protocol was made. It was heated only to see if the slower migration is merely an artifact of changing under heat - NO known or ligand added - set out at room temp w/ "anneal II"

Exp allowed to go O/N starting 7:15 pm
↳ 8:30 am

↓

Thursday

063

## Transformation Data

| | Ab | [stock] | [plate] |
|---|---|---|---|
| 2° Ab | AMP | 25 mg/ml | 50 μg/ml |
| | tet | 12.5 mg/ml | 12.5 μg/ml |
| | strep | 10 mg/ml | 25 μg/ml |
| | Chlor | 34 mg/ml | 10 μg/ml |

→ The 2° Ab were not added to the top agar—one in the master plate

### pUC19 DNA Dilutions

.5ng/plate ⇒ 3.8 ng 1:100,000

5ng/plate ⇒ 3.8 ng 1:10,000

50ng/plate ⇒ 38.5 ng 1:10,000

[Stock] = 13 μg/λ

### Anneal Mix

.5ng/plate ⇒ 1.5 ng 1:100

5ng/plate ⇒ 15 ng 1:100

50ng/plate ⇒ 1.5 ng unc

500ng/plate ⇒ 15 ng unc

### Media

B broth: 10 g bacto tryptone
8 g NaCl
add 1 liter $dH_2O$ & autoclave (liquid)

B plates: Add 15 g/λ Agar for plates

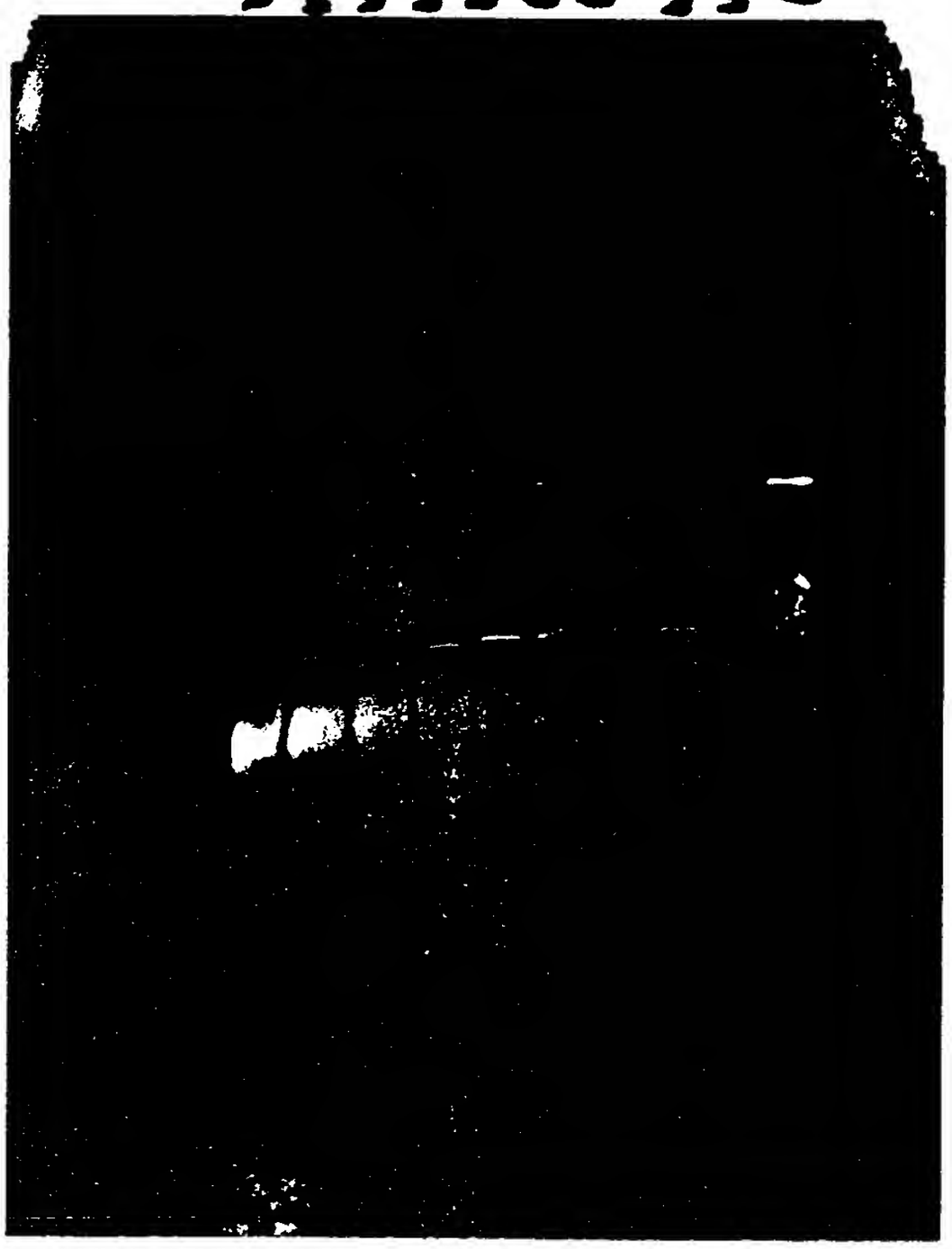
# Nomenclature for Replica Plating

W ⇒ white colony picked from PUCRAN plates
WB ⇒ blue colony " " " " "
B ⇒ blue colony picked from PUC19 plates

* The number following the above designation is arbitrarily assigned to the colony p

W☆ }
B☆ } ⇒ These colonies were picked from the plat according to the above nomenclature (minus the ☆), but they were NOT replica-plated. Because I worried for the plasmid prep that my liquid culture would not grow I picked a couple of colonies that would not "be replaced on the loop" by replica plating and used to inoculate liquid cultures (for the plasmid prep) only.

Qiaprep Miniprep from Transfected cells 070



The DNA was eluted in 20λ

4λ was run on the gel

CTO

Ward

KH HACH

5ml total

1 ml 5M NaOH
250 µl 20% SDS
3.75 ml $H_2O$
5 ml

40ml

.32g
4ml 10%
36ml $H_2O$

for 150ml 1.2g NaOH
7.5ml 20% SDS
142.5
150mls

fw NaOH = 40

20 ml Sol'n II

.16g NaOH
1ml 20% SDS
19ml $H_2O$
20ml total

.20g
1.25ml 20% SDS
23.75 ml
25ml

10% 1.09 g/ml
240ml
1.92g
80mls

.64g
8mls

72mls $H_2O$
80

Quick prep
Modified from Birnboim & Doly Nuc Ac Res 7, 1513-1523

## Quick plasmid (cosmid) preps.

Soln I = 50 mM Glucose
25 mM Tris pH 8
10 mM EDTA (pH 7.5)
1% mg/ml lysozyme (fresh) (for cosmids see over).

Soln II = 0.2 N NaOH
1% SDS (~~keep 1 week~~ make fresh!!!)

Soln III = 5 M KOAC pH 4.8 (60ml 5M KOAC + 11.5 ml HOAC → 100 ml).

1. Spin down 1 ml of o/n culture in Eppendorf tube 15 secs.
Remove all medium w. 1 ml Gilson. (If treating large nos. of samples, can use with drawn out pasteur pipette on a water-pump. Ditto for rest of protocol).
See over for 5 ml. cosmid cultures).

2. Suspend pellet in 100 µl Soln I with Eppendorf & vortexing.
(for cosmids, transfer to Eppendorf tube)
5 min at R. Temp.

3. Add 200 µl Soln II with Eppendorf. Mix by rocking tube sharply but don't vortex.
Inc. on ice for 5 min.

4. Add 150 µl precooled Solu III. Seal cap well, invert tube, mix gently on lid, vortex gently briefly, invert tube (to normal orientation); vortex gently briefly.

5. Inc. on ice/5 min.

6. Spin 1 min/microfuge.

7. Transfer supernatant (360 µl) to fresh tube, avoiding sediment.

~~7a add 1/2 DEP → incubate 65° for 10-15 min~~

8. Add 720 µl EtoH
Leave 2 min at R. temp.

9. Spin 1 min/microfuge.

10. Remove supernatant, carefully but completely with 1 ml Gilson.

11. Wash with 500 µl 70% EtOH, rinsing sides of tube with inclining tube (no vortexing or spinning)
Remove carefully but completely with 1 ml Gilson.

Dry (2 min/high vacuum).

Take up in 20 (50) µl TE, suspending pellet w Eppendorf.
Vortex, 4°/RT, vortex.

Before removing aliquots for digestion (~ 2 µl) (5 µl for cosmids)
Clarify by spinning 10 sec/microfuge.
~~Add RNase in digests. Can be scaled up preparatively. Used before PrOH.~~

Restriction Digests of QUICK plasmid preps

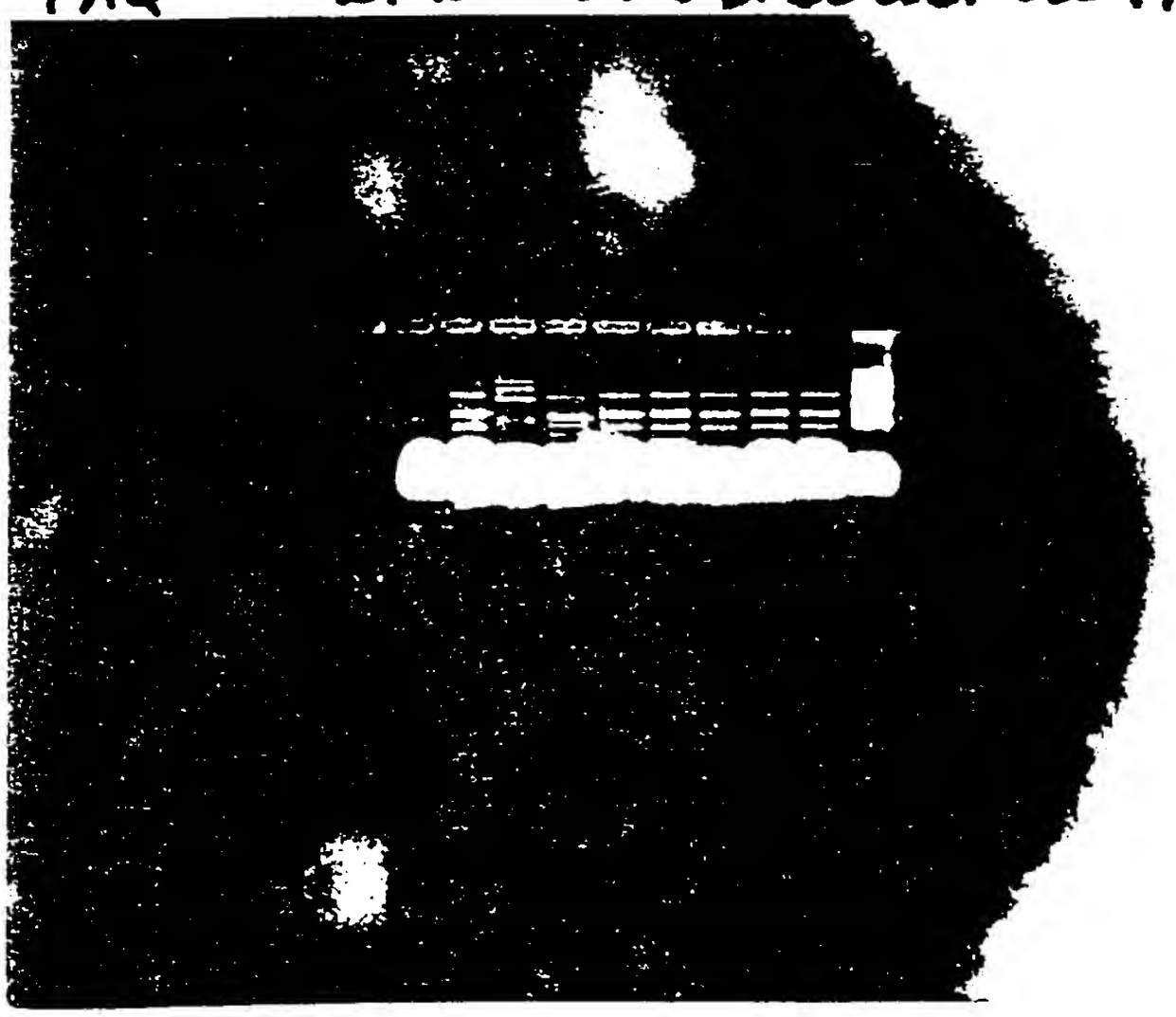
3λ of plasmid prep DNA
1.5λ of Taq I Restriction Endonuclease @ 10 u/λ
1λ of 10X core buffer
4.5λ of $H_2O$

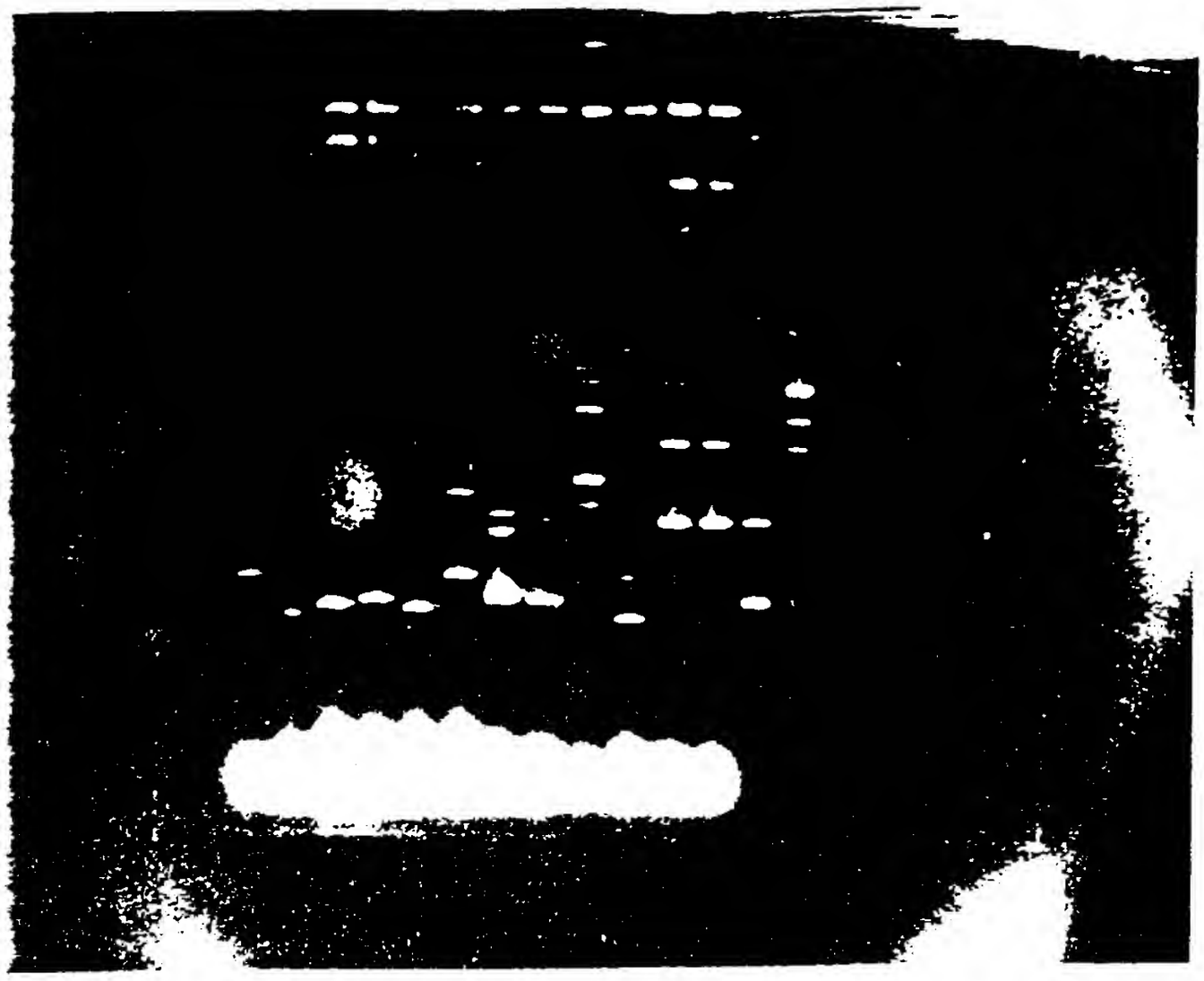
incubate at 37°C

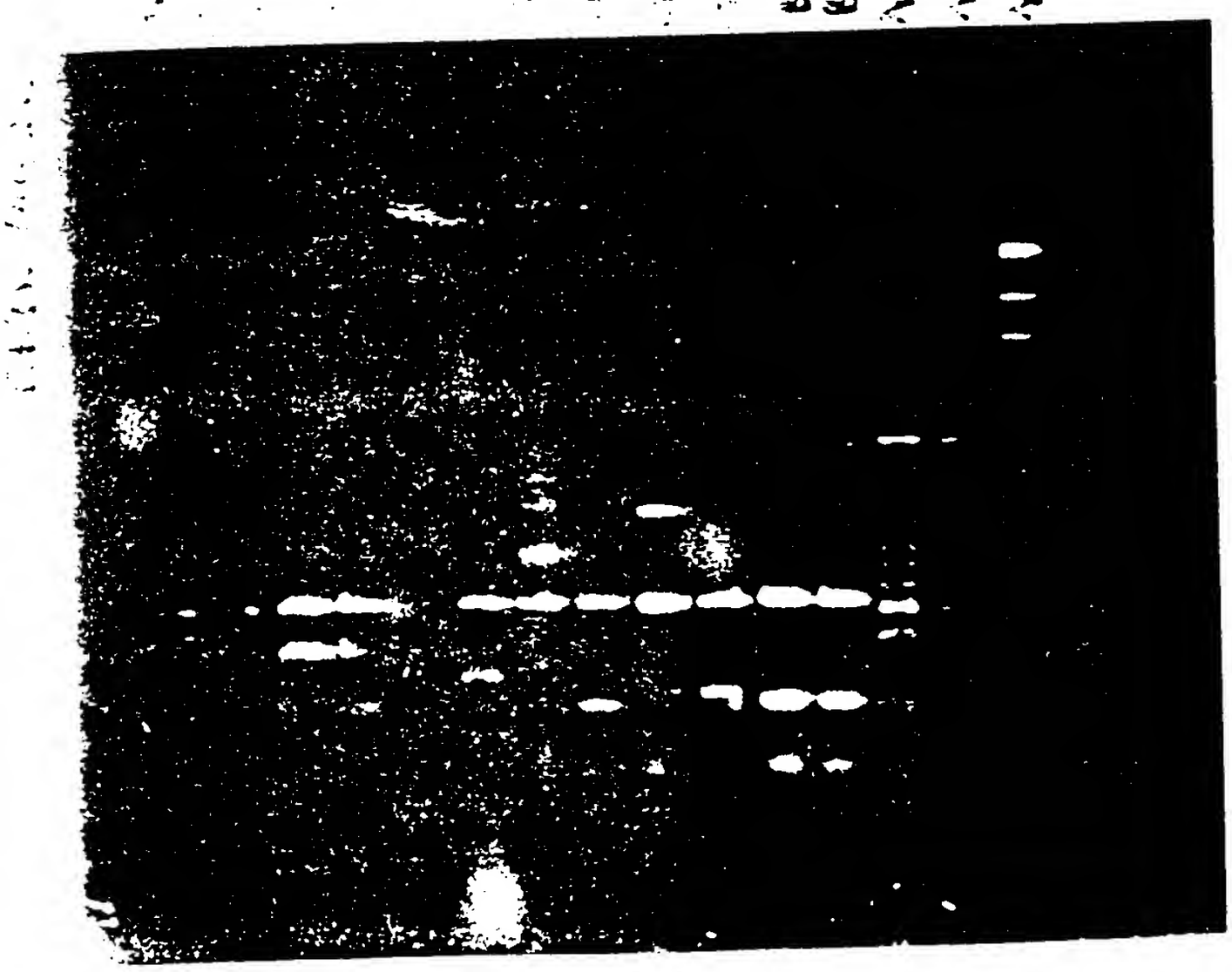
+ 19 stock digest
.3λ PUC19
1.5λ of Taq I
1λ 10X core buffer
7.2λ of $H_2O$

60min at 37°C

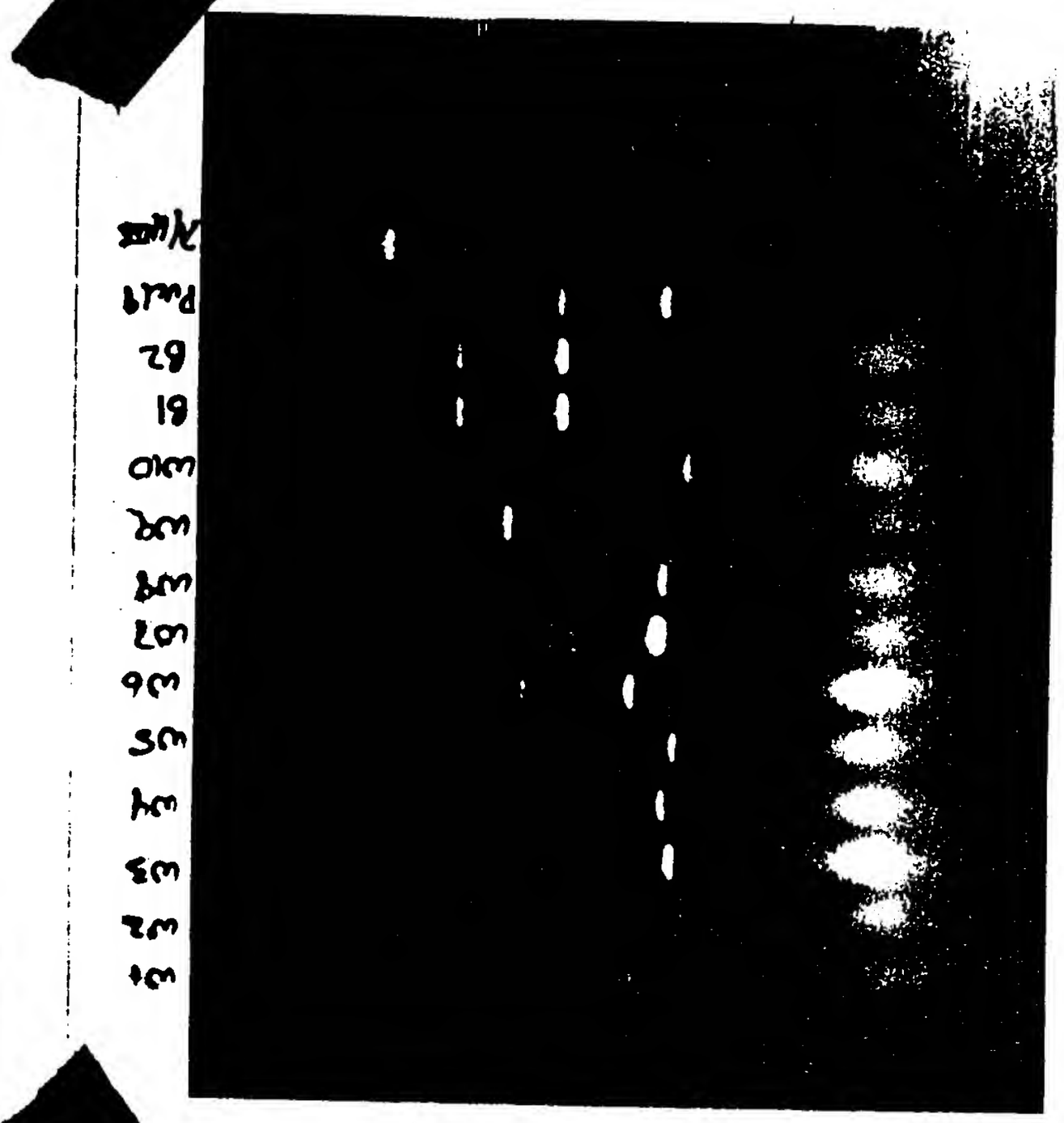
TAQ Digests W1·W2·W3·W5·B1·BR·W81·W82·19

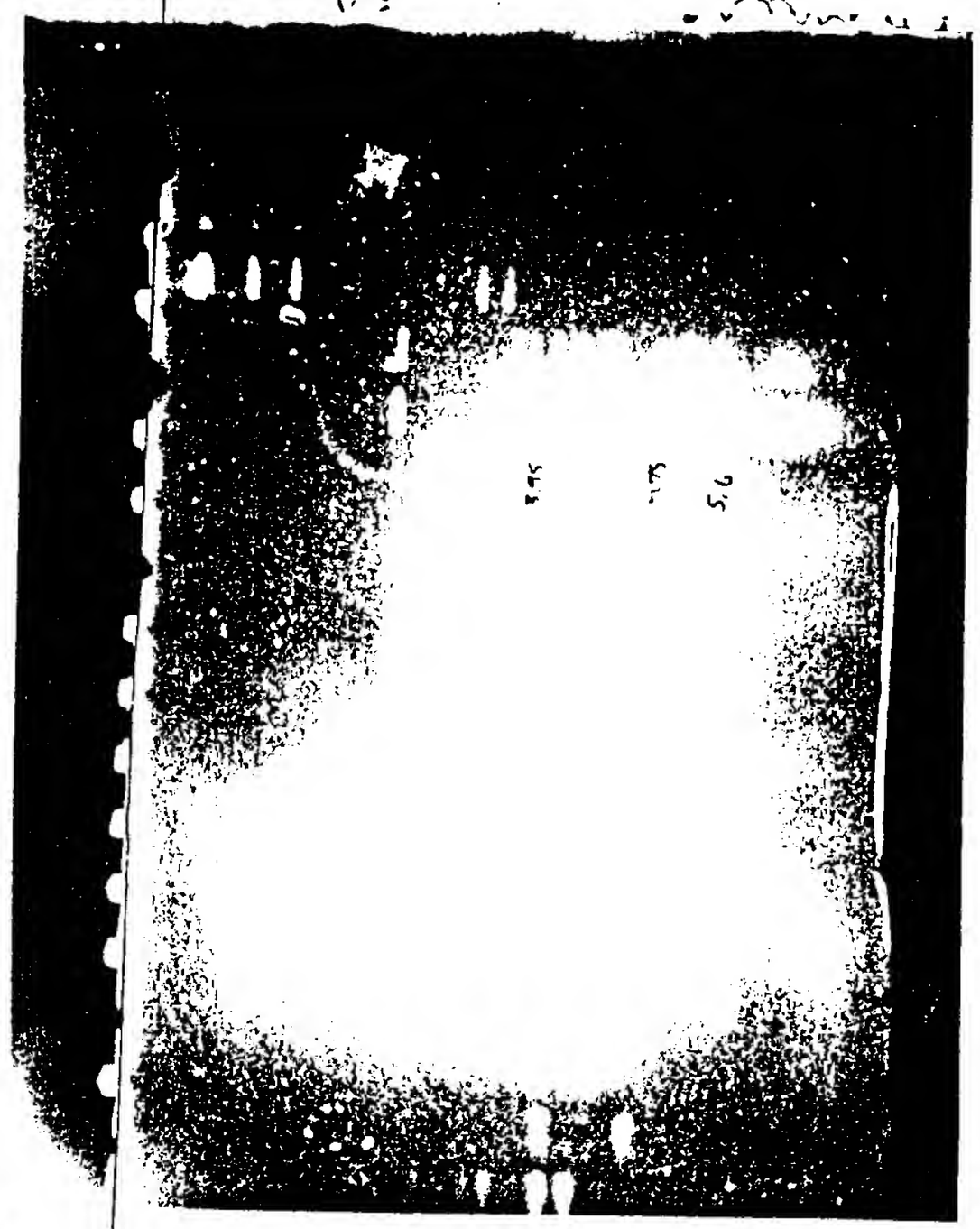



CUT
UNCUT



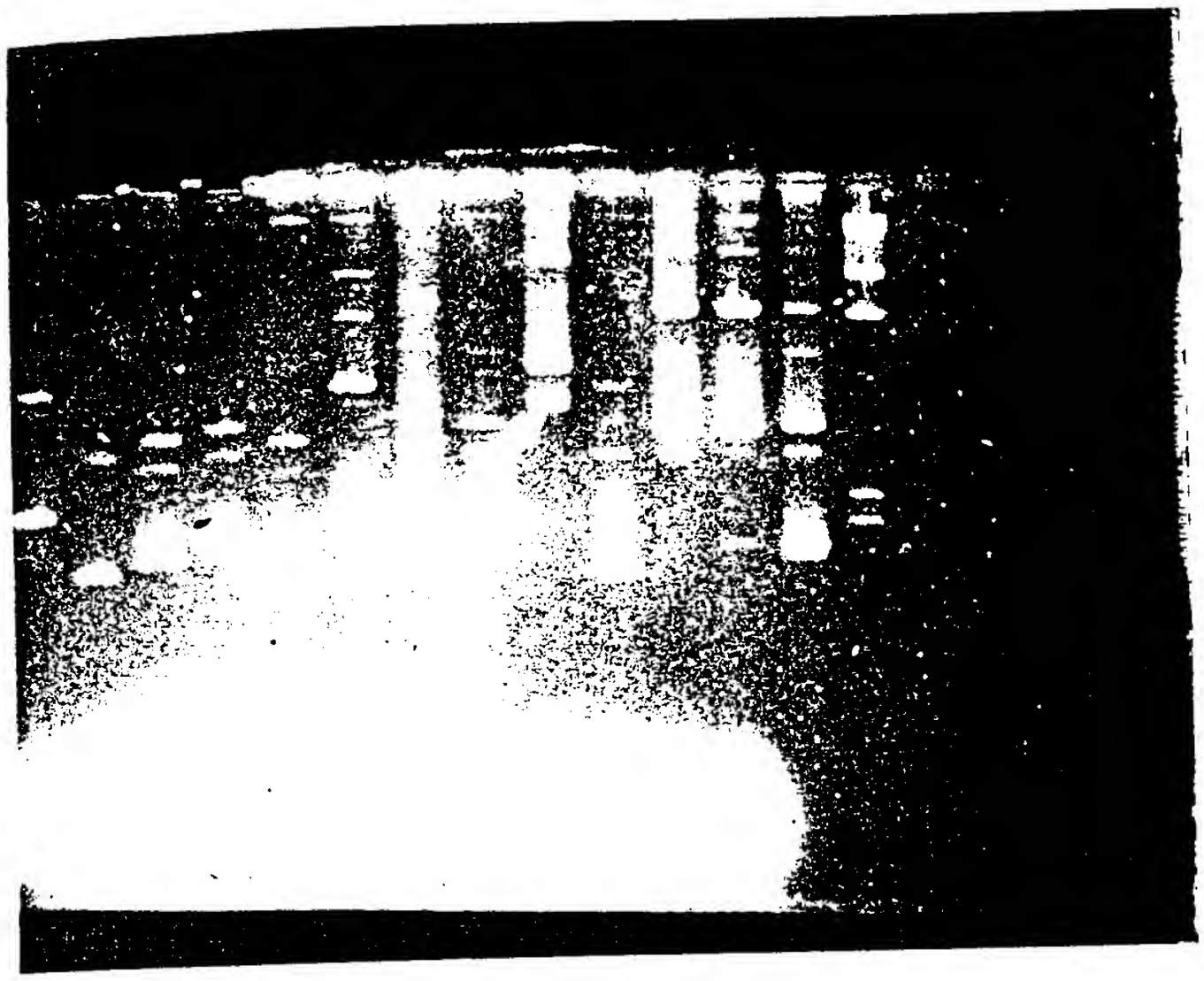
072


uncut
M/ucx
Pu19
62
61
uJO
uJQ
uJ8
uJ7
uJ6
uJ5
uJ4
uJ3
uJ2

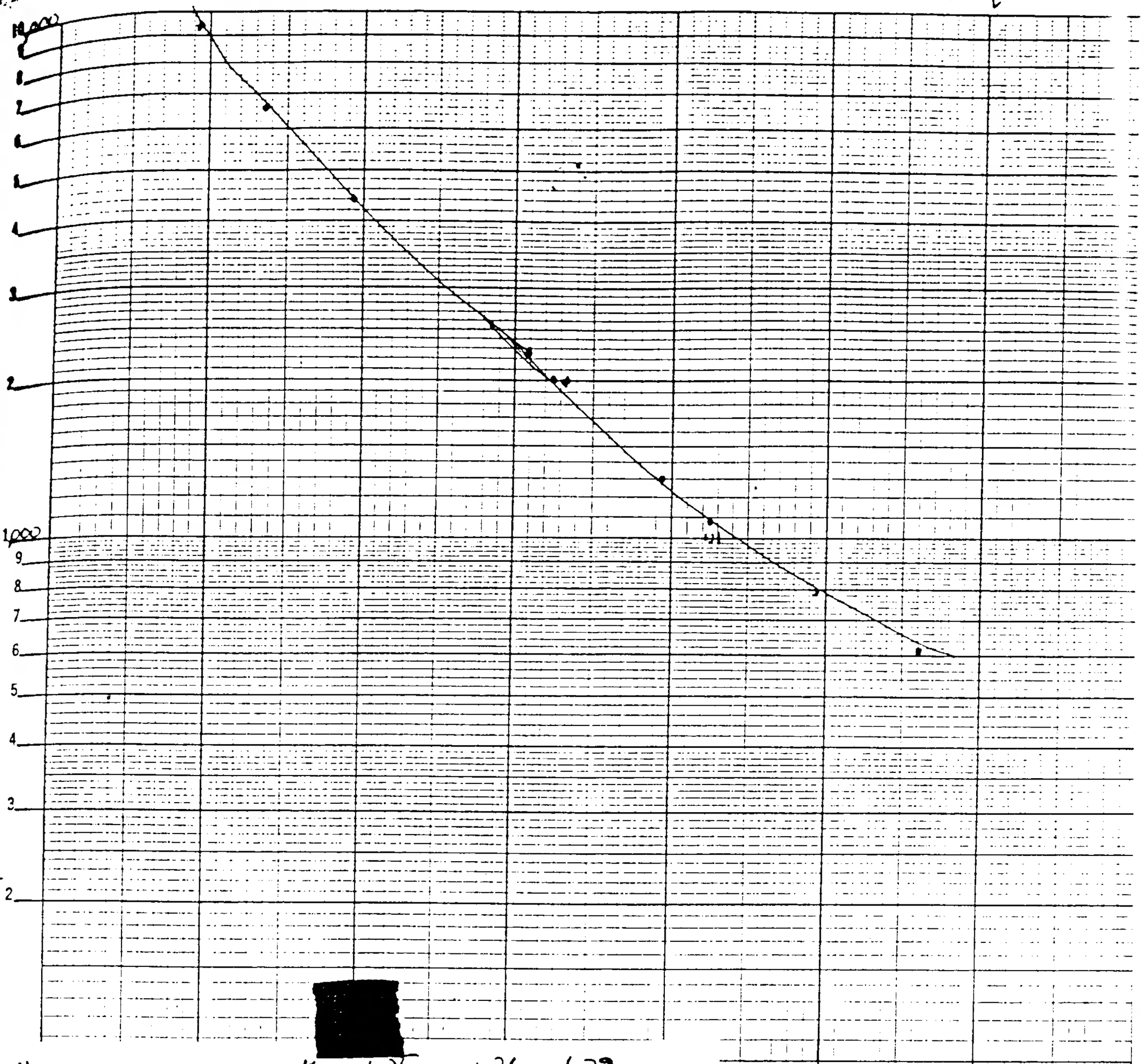

cut
12
5.6

073

Uncut



Standards from λ/HindIII
pUC19/TaqI




[redacted]

W1 — 2.5Kb, 2.0Kb, 1.3 Kb, 1.1 Kb

W2 — 1.3Kb, .85

W3 — 1.3, 1

W4 — 1.3, .8, .6

W5 — ϕ

W6 — 1.3, .9, .6

W7 — 2.2Kb, 1.3Kb, .5Kb

(short & fast calculation of size of bands)

[redacted]

: [redacted] growth after [redacted] [redacted]
tried:

| | | |
|---|---|---|
| fox-5 | 19 g/ml | 1.9 λ stock |
| fox-6 | 8.5 g/ml | .9 λ stock |

| | sulf | trimeth | |
|---|---|---|---|
| ST-5 | 5 g/ml | 1 g/ml | 357 λ @ 1:10 stc |
| ST-6 | 2.5 g/ml | .5 g/ml | 1.87 λ @ 1:10 stc |

FOX4-A has growth (but FOX3-A does not, while all others foxes do) except for FOX4-19 & FOX3-19. (From 9-25 → 9-27 these were allowed to sit at room temp.)

3 (1 ML) pairs of FOX4-T was withdrawn & pelleted; SN decanted & pellet frozen at -70°C (Stored in Ray's "In Use" box) to save for future plasmid prep.

100 ml of FOX4-A was dumped onto a ZKyT plate and AB sensitivity discs applied.

From the 2° plates (STX & FOX) all colonies were lifted (one at a time w/ a loop) and added to 2.5 ml liquid ZKyT, spun down overnight at 4°C, and then 100 ml of each was dumped onto a ZKyT plate

## Selection Experiment

Goal: To see if any of the pUCRAN DNA will confer drug resistance

Methods:

JM83 was transformed with pUCRANIIDNA≡"AII" @ 300 ng per plate (totaling 2 plates) according to the protocol on p. 66y (Control: pUC19 @ .5ng (AII → ~750 white colonies; pUC19 ~1000 blues)

"lifts" → On [redacted] 6ml of 2xYT liquid was added to plate - swished around and raked (w/ glass rake). 200λ of this infective liquid was plated onto large 15mm Petri AMP, 2xYT plates (AMP @ 50λ/ml). Antibiotic disks were added to the plate (~30 different ones). There were a total of 4 plates: 2 "lifts" from pUC19 controls (1 not shown above) and 1 each from the two AII(≡ pUCRANII) DNA. (The II merely denotes the second batch prepared according to the protocol on p. 65y).

diameters of [redacted]

| [redacted] | | PVC19#1 | PVC19#2 | ATT#1 | ATT= |
|---|---|---|---|---|---|
| ofurantoin | F/M | 2cm | 2.2cm | 2cm | |
| olfamethazol | TH | Φ | Φ | Φ | |
| lfamethazide trimethoprim | SXT | 1 1cm | 1.6cm | Φ | |
| mpicillin | AM | Φ | Φ | Φ | |
| tracycline | TE | 2cm | 2.3cm | 2.1 | 2 |
| olymixin B | PB | 1.2cm | 1.2cm | 1.2 | 1.2 |
| | VN | 1cm | 1.1cm | 1.1 | 1.1 |
| efuradol | MA | Θ | Θ | Θ | Θ |
| | CF | Θ | Θ | Θ | Θ |
| ticarcillin | TIC | Θ | Θ | Θ | Θ |
| entamicin | GM | 1.2cm | 1.3 | 1.1 | 1.2 |
| moxalactam | MOX | 2.7cm | 2.7 | 2.9 | 2.5 |
| | FOX | 2.4cm | 2.4 | 2.4 | 2.4/1cm given |
| Cefotaxime | CTX | 2.5cm | 2.6 | 2.7 | 2.6 |
| pipericillin | PIP | Φ | Φ | Φ | Φ |
| [redacted]cefoperazone | CFP | Φ | Φ | Φ | Φ |
| | AN | 1.5cm | 1.5 | 1.4 | 1.4 |
| chloramphenicol | C | 2.7cm | 2.7cm | 2.4 | 2.4/1cm |

sulfamethoxazole trimethoprim = SXT
cefoxitin = fox 30

ST-A colonies picked from single colonies growing around SXT discs on 2° plates

ST-19 colonies
& FOX-19 colonies > picked from lawn on big 19-plates

FOX-+ colonies picked from single colonies on 2° plates grown in absence of fox (but incubated first)

Liquid broths

Cefoxitin stock

50 mg/ml
= .05g x 10ml = .5g in 10ml

Each tube: 5ml 2xYT
10λ of 25mg/ml AMP ⇒ 50g/ml

| | Cefoxitin | (AMP is labelled 10 MCG from disk package; FOX is labled 30 mcg) |
|---|---|---|
| fox-1 | 37.5g/ml | → 3.8λ of stock |
| fox-2 | 75g/ml | 7.5 λ stock |
| fox-3 | 150g/ml | 15λ g stock |
| fox-4 | 300 g/ml | 30λ g stock |

sulfamethoxazole trimethoprim

stock: 16mg/ml trimeth
80mg/ml sulfa

| | Sulf | Trimeth | |
|---|---|---|---|
| ST-1 | 10g/ml | 2g/ml | .7λ g stock |
| ST-2 | 20g/ml | 4g/ml | 1.3λ g stock |
| ST-3 | 40g/ml | 8g/ml | 2.5λ g stock |
| ST-4 | 80 g/ml | 16g/ml | 5λ g stock |

for Δβ disc sensitivity testing.

In going through old plates a single colony was noticed on a month old TET plate, it was picked & grown 2 days in air shaker at 37°C (in 12.5 &/ml 6.3 &/ml, 3.2 &/ml, and 1.6 &/ml [TET] against controls)

For all Pic19 controls, a loop was dipped into an area of the lawn on the very large, 1° Δβ sensitivity plates (away from the discs)

liquid
~~Colonies~~ picked for plasmid preps

TET @ 1.6 &/ml + 50 &/ml AMP — 2 days 3?
2 days R?

19 @ 50 &/ml AMP — 2 days 37°C — TET from plate
+ 5 &/ml SULF — 2 days RT
1 &/ml trimeth

SXT @ 50 &/ml AMP — 2 days 37°C — Selected
+ 5 &/ml SULF — 2 day RT — 2° plate
1 &/ml trimeth

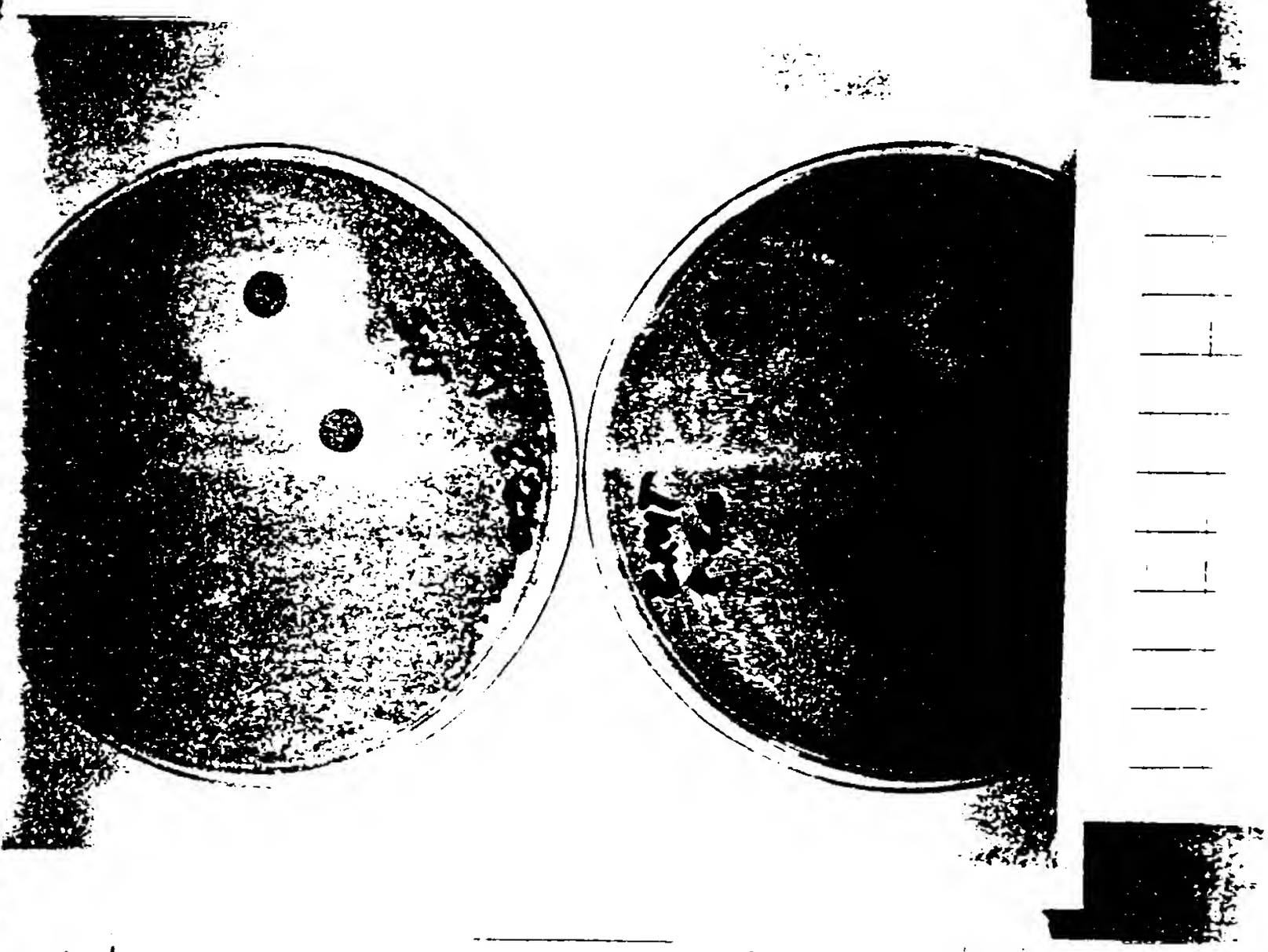
SXT @ 50 &/ml AMP — 2 days 37°C — Selected
80 &/ml SULF — 2 days RT — 2° plate
16 &/ml trimeth

FOX @ 50 &/ml AMP — 2 days 37°C — unselected
200 &/ml cefoxitin — 2 days RT — 2° plate

# Pursuit of SXT resistance

## colony isolation

The SXT[res] colonies from the original screening were plated onto 2° ampKan[res] grid plates. All colonies from this 2° grid were picked and inoculated into 2ml 2x~ liquid. 100 λ of this inoculum was spun onto a 2xYT plate and checked for AMP & SXT sensitivity w/ the ΔB paper discs. A pattern similar to the original screening was observed. (The photo below is a picture of second screening plate)



A loop taken from as close to the paper disc as possible was smeared onto an LAMP plate to isolate single colonies. Single colonies were isolated and grown on an LAMP grid plate in which the agar pricks were made around the edge of an SXT ΔB paper discs. These are then isolated single SXT[res] AMP[res] colonies (PUC19 prepared as above — colonies picked from the lawn on the original selective plate — did not grow at all near the SXT discs).

# ← see p. 74

The above single colonies were used in the following liquid inoculum:

Each tube: 5ml 2XYT
50 µl/ml AMP

| | Sulf | trimeth | |
|---|---|---|---|
| ST-4 | ~~80~~ 80 µl/ml | 16 µl/ml | 5 µg stock |
| ST-3 | 40 | 8 | 2.5 µg stock |
| ST-2 | 20 | 4 | 1.3 µg stock |
| ST-1 | 10 | 2 | .7 µg stock |
| ST-0 | ⊖ | ⊖ | ⊖ |

following the label
an "A" denotes putative STres and an "19" indicates puc19 control.

The colonies were inoculated into the 2XYT+A and allowed to grow for 1 hour 15 minutes before the SXT was added.

Puc19 controls are from single colony Puc19's on an AMP plate

results

| | A | 19 |
|---|---|---|
| ST-4 | ⊖ | ⊖ |
| ST-3 | + | + |
| ST-2 | + | + |
| ST-1 | ⊖ | + |
| ST-0 | + | + |

⊖ = NO
+ = grow

ST-4 A & ST-019 ~~pulled~~ pulled for plasmid preps

preps digested!

3λg DNA
1λg 10X cutsmart buffer
1.5λg Taq I 5 U/ul ~~BSA~~
2λg 10% Triton X-100 (1:100 dilution stock) RNase
2.5λg $H_2O$
10λ total

65°C 2½ hours

65°C @ 12:00 pm

8:35 am